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COMMENTARY

The Methodologic Dilemma in Retrospectively Correlating the Amount of Chemotherapy Received in Adjuvant Therapy Protocols With Disease-Free Survival[1,2]

Carol Redmond, Bernard Fisher, and H. Samuel Wieand[3,*]

When carrying out a study to evaluate the worth of a chemotherapeutic regimen it is important that the amount of drug received by the patients is consistent with that prescribed by the protocol. For various reasons a substantial number of patients may not have received the quantity planned. As a consequence, the study may be rejected, since conclusions cannot be formulated regarding the value of the treatment. On the other hand, the results may be accepted as found and considered representative of what would be expected if the regimen were to be employed on a population as a whole. Alternatively, an attempt may be made to obtain, retrospectively, information which could be used to relate the amount of drug received by patients to their outcome even though that correlation was not one of the original aims of the investigation.

Recently, a retrospective analysis was made of patients who had been entered into a prospective randomized trial to evaluate the worth of cyclophosphamide, methotrexate, and 5-FU (CMF) as adjuvant therapy for breast cancer. A report of that analysis indicated that the disease-free survival of patients was directly related to the amount of drug received (1). Other investigators also considered their findings according to the proportion of the protocol dose which was received (2–4). We have attempted to correlate the amount of drug received with disease-free survival by retrospectively examining information from two National Surgical Adjuvant Breast and Bowel Project (NSABP) trials in which adjuvant therapy was administered for 2 years (5,6). That undertaking has emphasized the methodologic difficulties encountered when making such an analysis and the hazard of drawing conclusions from the findings.

THE PROBLEM

A number of serious methodologic problems arise that make this type of post hoc analysis difficult to perform. When contemplating such a retrospective evaluation, and *prior* to analyzing the information obtained, it is imperative that several major issues be resolved. The first is to decide upon the appropriate manner in which to summarize the amount of drug that the patient has received. Should one accumulate the total amount given in all of the courses and use that value, or should one convert it into a percentage of the maximum contemplated protocol dose and employ that measure in the correlation with patient outcome? What should be done when several different drugs are given in combination? Should the doses of each of the drugs be combined into a single dose to arrive at an overall measure of "dose" given, or should a weighted average of each of the drugs be employed? When a decision is reached on how best to determine the amount of drug that the patient has received, it must then be decided how best to correlate that amount with treatment failure and survival of patients. Should they be stratified according to dose levels, and how might those levels be selected? Should the results be examined from the time therapy was initiated, or should they be evaluated only subsequent to completion of therapy? Are there statistical approaches which enable one to take into account the cumulative nature of the amount of drug received?

POTENTIAL METHODS FOR RESOLVING THE PROBLEM

Several definitions of the dose of drug(s) that a patient has received may be considered.

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[3]National Surgical Adjuvant Breast and Bowel Project Headquarters, University of Pittsburgh, PA.

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Method I

The first method calculates the amount of drug taken by each patient, without consideration of any of the factors which may have led to a reduction or cessation of the therapy:

$$\text{amount of drug received (\%)} = \frac{\text{total of all drug received}}{\text{maximum protocol dose (full protocol dose at each course} \times \text{protocol No. of courses)}} \times 100.$$

The following indicates how the use of this most obvious approach for determining amount of drug received produces biased results. Using data from NSABP protocols, the amount of melphalan [L-PAM (P)] or P + 5-FU (PF) received was calculated. Patients, whether receiving P or PF, were grouped into three levels according to whether they received ≥ 85% (Level I), 65%–84% (Level II), or < 65% (Level III) of the protocol-stipulated dose of P. The level of P and of F was found to be similar in PF-treated patients, eliminating the need for averaging the proportion of the two drugs received. Life tables were then constructed for each of the groups. The distribution of patients at each dose level and the life-table disease-free survival at 5 years for those receiving P (table 1) and at 4 years for those receiving PF (table 2) are shown. When P was given, there was little difference in the proportion of the patients ≤ 49 or ≥ 50 years old (about 35%) who received the maximum (Level I) amount of drug. With PF, more of the patients ≥ 50 than ≤ 49 years old received the Level I amount. Only 6% of the older patients got < 65% of the protocol dose, while 30% of the younger women were in the Level III group, indicating that the older women tolerated the drug as well as, if not better than, did the younger age group.

The data demonstrate a consistent difference in disease-free survival among P- or PF-treated patients at the three dose levels. This was observed whether they were considered overall or according to age and nodal status. Increasing dose was associated with increasing disease-free survival. In some instances Level III was associated with a poorer disease-free survival than that observed in the control group. At times, the difference was quite marked. This observation is exemplified by patients ≥ 50 years of age with 1–3 positive nodes. Those receiving < 65% of P had a disease-free survival which was 24% lower than that for the untreated control patients. Since the overall results in that subgroup, without regard for dose received, indicate no difference between treated and nontreated patients, the disparity observed in patients receiving the Level III amount of drug must of necessity be associated with a compensatory benefit in those getting the Level I amount. In this example, acceptance that more drug is associated with a benefit requires that there be a similar acceptance that the least amount of drug is harmful. Consequently, the presence of a significant dose/response relationship in an otherwise negative comparison of drug-treated patients to controls, as in this group of patients, poses difficulties in interpretation.

The greater disease-free survival in Level I patients is due to the fact that those who failed early were automatically excluded from this group because protocol treatment is stopped at failure; they were not receiving therapy long enough to have accumulated ≥ 85% of the protocol amount, even if they had received full doses up to the time of recurrence. The patients with early failures, therefore, concentrate in the low-dose groups and thus account for the decreased probability of disease-free survival in those given the Level III amount of drug. The bias produced by this method of determining drug dose

TABLE 1.—% disease-free survival at 5 yrs, by % dose level for P (Method I)

Age and nodal status	Dose level* I (≥ 85%)	II (65%–84%)	III (< 65%)	Any dose*	Control*	P value†
All patients	69	67	26	51	46	< 10-5
	(182)	(140)	(201)	(525)	(505)	
≤ 49 yrs	71	80	20	52	37	< 10-5
	(77)	(46)	(79)	(203)	(170)	
1-3 positive	73	92	29	66	51	< 10-5
	(53)	(25)	(29)	(108)	(86)	
≥ 4 positive	69	66	15	37	23	< 10-5
	(24)	(21)	(50)	(95)	(84)	
≥ 50 yrs	67	61	30	51	50	< 10-5
	(105)	(94)	(122)	(322)	(335)	
1-3 positive	77	71	41	63	65	< 10-5
	(75)	(50)	(46)	(171)	(168)	
≥ 4 positive	42	48	24	35	36	< 10-3
	(30)	(44)	(76)	(151)	(167)	

* Values = % disease-free patients (total No. of patients).
† I vs II vs III (Mantel-Cox test based on life table).

TABLE 2.—% disease-free survival at 4 yrs, by % dose level for PF (Method I)

Age and nodal status	Dose level* I (≥ 85%)	II (65%-84%)	III (< 65%)	Any dose*	Control*	P value†
All patients	78 (311)	63 (166)	39 (207)	63 (689)	49 (505)	< 10-5
≤ 49 yrs	75 (120)	65 (71)	40 (89)	62 (284)	41 (170)	< 10-5
1-3 positive	89 (75)	74 (44)	65 (40)	77 (160)	56 (86)	0.009
≥ 4 positive	50 (45)	49 (27)	20 (49)	40 (124)	26 (84)	< 10-5
≥ 50 yrs	80 (191)	61 (95)	38 (118)	63 (405)	53 (335)	< 10-5
1-3 positive	83 (107)	73 (55)	44 (47)	72 (209)	68 (168)	< 10-5
≥ 4 positive	75 (84)	48 (40)	34 (71)	54 (196)	38 (167)	< 10-5

*Values = % disease-free patients (total No. of patients).
†I vs II vs III (Mantel-Cox test based on life table).

may be vividly demonstrated by examining the relationship between the dose of a placebo received and patient outcome (fig 1). Women receiving the greatest amount of placebo (Level I) had the best disease-free survival, while those getting < 65% of the placebo had a significantly poorer one.

With this method of dose determination, the longer the time of therapy specified by the protocol, the greater the magnitude of the bias. For example, a patient having a treatment failure any time within the first year of a prescribed 2 years of treatment cannot possibly receive Level I therapy; hence, the Level I group will contain no patients having treatment failures within the first year. If the protocol had stipulated that therapy be given for only 1 year instead of 2, patients who failed in the latter part of the first year may have received Level I therapy. The length of time that a patient must continue therapy to be classified in Level I is directly proportional to the prescribed length of the therapy.

Thus, for patients who had an early termination of therapy because of treatment failure or other events such as an early death, which precluded continuing therapy, it is inappropriate to base the maximum dose (denominator) on the full number of courses as defined by the protocol.

Method II

The following definition may seem to provide a logical method to correct for the bias observed with the first approach:

$$\text{amount of drug received (\%)} = \frac{\text{total of all drug received}}{\text{maximum protocol dose possible prior to treatment failure or death}} \times 100.$$

The results with this method differ from those noted when Method I was used. In general, patients receiving higher dose levels of P or PF demonstrated the poorest disease-free survival. With the use of P, patients receiving Levels II or III fared somewhat better than did those receiving the highest dose (Level I) (table 3). There was also a tendency for the PF-treated patients receiving Level III to have the best disease-free survival (table 4), even when Level I therapy was found to be beneficial. Thus, with the use of this approach for determining drug dose, it cannot be concluded that increasing amounts of drug are associated with better disease-free survival.

A major bias introduced with this type of analysis is that patients who failed early would be more likely to have received ≥ 85% of the protocol dose, since they would not yet have experienced toxicity sufficiently profound to have resulted in marked dose reductions. As patients progressively approach completion of therapy, the more likely it becomes that they have required a number of dose reductions because of episodes of toxicity, so that they receive a lesser percent of the protocol-prescribed dose. In addition, as the time of treatment progresses, the greater becomes the likelihood of noncompliance. This approach for obtaining the percent of drug received and for relating that percent to disease-free survival introduces a bias which is opposite in direction to that introduced by Method I. The use of a placebo group further illustrates the bias by this method, although the differences noted are not as strikingly significant because placebo does not produce toxicity (fig 1). Noncompliance, rather than toxicity, is the important factor introducing this bias when a placebo is employed.

Since neither of the two approaches results in an interpretable analysis, they are both inappropriate. The design of an appropriate analysis is hampered by a variety

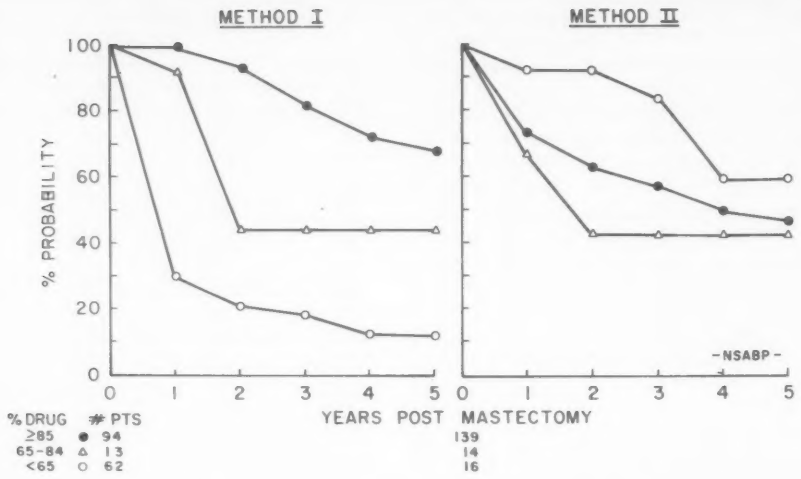

FIGURE 1.—% probability of disease-free survival related to dose level of placebo: Methods I and II.

of temporal factors which influence the amount of therapy received. That therapy is not administered as a single dose at one point in time and that one or more drugs are given repetitively during a prolonged time interval pose problems. During that period, physician or patient noncompliance, intercurrent disease, early treatment failure, early death, and other factors may interfere with the therapy as planned. The amount of toxicity requiring drug dose reduction also tends to be time-related in that it increases in frequency and degree as the time on therapy progresses.

Aside from the time-related factors, other factors such as age, degree of nodal involvement, and tumor characteristics, all of which influence the natural history (prognosis) of the disease, may also affect the amount of drug received. For example, if it is decided at the start of a study to give older patients less of a therapeutic agent than is given to those who are younger because it is thought that they would be unable to tolerate as much drug, there will result a difference in that characteristic (age) between the groups receiving higher or lower doses. This type of confounding of prognostic factors must be considered in any analysis based upon such nonrandomized comparisons. Further, a difference in patient health status or other characteristics may covertly influence the physician to employ less than a "full" dose, leading to a selection bias in any dose-response comparison.

TABLE 3.—% disease-free survival at 5 yrs, by % dose level for P (Method II)

Age and nodal status	Dose level* I (≥ 85%)	II (65%–84%)	III (< 65%)	Any dose*	Control*	P value†
All patients	47	59	55	51	46	0.005
	(245)	(151)	(127)	(525)	(505)	
≤ 49 yrs	46	75	46	52	37	0.006
	(106)	(49)	(47)	(203)	(170)	
1-3 positive	61	89	51	66	51	0.05
	(56)	(28)	(23)	(108)	(86)	
≥ 4 positive	31	56	41	37	23	0.03
	(50)	(21)	(24)	(95)	(84)	
≥ 50 yrs	48	50	60	51	50	0.06
	(139)	(102)	(80)	(322)	(335)	
1-3 positive	62	70	71	63	65	0.36
	(85)	(53)	(33)	(171)	(168)	
≥ 4 positive	24	29	52	35	36	0.001
	(54)	(49)	(47)	(151)	(167)	

* Values = % disease-free patients (total No. of patients).
† I vs II vs III (Mantel-Cox test based on life table).

TABLE 4.—% disease-free survival at 4 yrs, by % dose level for PF (Method II)

Age and nodal status	Dose level* I (≥ 85%)	II (65%–84%)	III (< 65%)	Any dose*	Control*	*P* value†
All patients	60	65	70	63	49	0.01
	(408)	(161)	(115)	(689)	(505)	
≤ 49 yrs	57	67	70	62	41	0.02
	(160)	(69)	(51)	(284)	(170)	
1–3 positive	79	71	92	77	56	0.22
	(84)	(46)	(29)	(160)	(86)	
≥ 4 positive	31	58	44	40	26	0.04
	(76)	(23)	(22)	(124)	(84)	
≥ 50 yrs	63	64	71	63	53	0.28
	(248)	(92)	(64)	(405)	(335)	
1–3 positive	74	73	66	72	68	0.94
	(121)	(55)	(33)	(209)	(168)	
≥ 4 positive	51	54	75	54	38	0.11
	(127)	(37)	(31)	(196)	(167)	

* Values = % disease-free patients (total No. of patients).
† I vs II vs III (Mantel-Cox test based on life table).

Method III

To surmount the problem caused by treatment failures occurring while patients are on therapy, the dose received up to a specific time may be related to the disease-free survival and survival subsequent to that time. If a specific time during therapy is selected for analysis of the amount of drug given, a problem arises in that the amount received after that time may also influence the end results. When a time is chosen at or after completion of the therapy that difficulty obviously does not occur, but another exists. A serious limitation of the method is that it utilizes only patients who have completed therapy without a treatment failure. As an example of this approach, disease-free survival was determined only in patients who had completed 2 years of PF therapy as stipulated by the protocol (fig 2). Patients who failed to receive the complete therapy because of treatment failure were not included. The disease-free survival was evaluated relative to the amount of drug received during the 2-year period. Variation in the amount of drug received failed to influence treatment failure either in all patients or in the two age groups. This was observed when either the melphalan or the 5-FU in the PF combination was evaluated separately or in combination. It is emphasized that these life-table plots do not present the overall response to the therapy but relate only to the disease status of patients subsequent to completion of 2 years of therapy.

While this method is not subject to the temporal biases in the first two methods, it provides a less than complete analysis of the results because of the omission of patients failing while receiving therapy. This is particularly serious, since the most striking differences in disease-free survival occur during that period of time.

Method IV

A method which is useful for considering the relationship between the amount of drug received and treatment failure includes information from all patients, even those who failed while on therapy. To evaluate the effectiveness of each dose level of chemotherapy received, the percent of protocol dose received is calculated for each patient to the time that a treatment failure occurs. With information on the distribution of doses received by all patients at risk of failing, it is possible to estimate a coefficient that represents a linear dose effect. This approach based upon the Cox model (7) also permits adjusting for other prognostic factors that may influence outcome. The methodology is well-described in recent statistical literature (8) and is employed extensively in the analyses of results from biomedical research. In our analyses the form of the regression model employed assumes a hazard function of the form:

$$\lambda(t) = \lambda_0(t) \exp [Z_1\beta_1 + Z_2\beta_2 + Z_3(t)\,\beta_3],$$

where $Z_1 = 0$ for age ≤ 49
$= 1$ for age ≥ 50
and $Z_2 = 0$ for 1–3 positive nodes
$= 1$ for ≥ 4 positive nodes
and $Z_3(t)$ is the cumulative dose at time t.

Note that with this method the amount of drug received is recalculated for each patient at the time of *each* treatment failure.

The analysis employed by us addresses several questions. Is there any evidence during the entire follow-up period that there is a relationship between the percent dose received and disease-free survival? Is the relationship between percent dose received and disease-free sur-

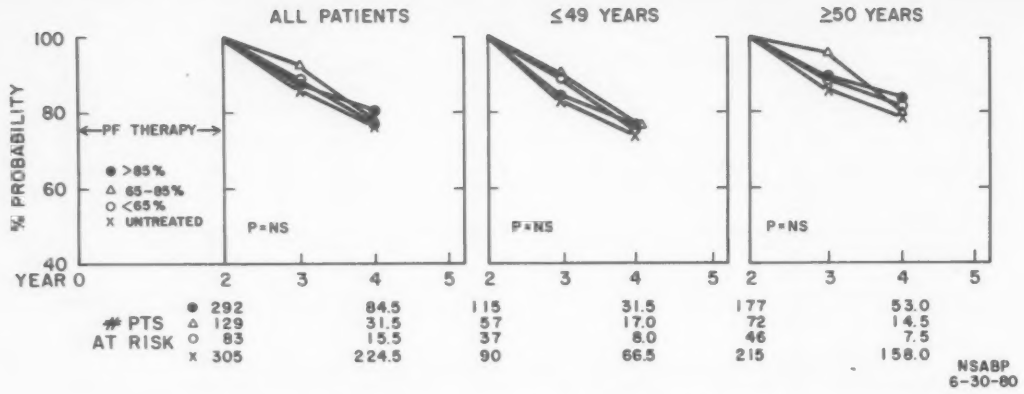

FIGURE 2.—Comparison of amount of therapy and disease-free survival subsequent to completion of therapy: PF.

vival most apparent during the time when chemotherapy is being received or only after the cessation of chemotherapy? Finally, does any reduction in dose immediately preceding a failure have an effect upon any apparent dose-response relationship? Information was sought to ascertain what influence the last course of therapy has in the analysis of the dose-response relationship. It was noted in some instances that patients who demonstrated a treatment failure did not receive their last scheduled course of therapy prior to the specific documentation of recurrent disease. For example, a patient presents for a scheduled course (dose) of chemotherapy and, as a result of clinical evaluation, it is decided to delay drug administration until it is determined whether or not the patient has a recurrence. If it is decided that she does have a treatment failure, the protocol therapy is discontinued. She may be documented as being noncompliant relative to her last course of therapy. To address this question, percent dose was based on all chemotherapy received up to 2 months prior to the documentation of a recurrence. If a dose-response relationship is primarily related to the amount of therapy received in the period immediately preceding a treatment failure, evidence for a cause and effect relationship is tenuous since the dose reduction may be the result of the recurrence rather than vice versa.

The findings from two analyses are presented. In the first (table 5), the percent dose was calculated for each patient at risk based upon all courses the patient should have received up to the time at which a recurrence was documented. In the second (table 6), the percent dose was based on all courses the patients should have received up to 2 months prior to the documentation of a treatment failure. In all analyses, adjustment has been made for age ($\leq$ 49 or $\geq$ 50 years) and number of positive nodes (1–3, 4–9, 10–14, or $\geq$ 15).

The results of the regression analysis relating the proportion of drug received to disease-free survival indicate that patients receiving PF therapy show some trend toward having a better disease-free survival during the 2 years of chemotherapy, if one considers all therapy up to

TABLE 5.—Cox regression analysis relating proportion of drug received to disease-free survival (overall)

Time interval	No. of patients	Coefficient	Standardized coefficient	*P* value*
Placebo patients				
All follow-up	168	-0.737	-1.947	0.05
0–2 yrs	168	-0.752	-1.499	0.14
> 2 yrs	102	-0.721	-0.721	0.19
PF patients				
All follow-up	648	-0.517	-1.759	0.08
0–2 yrs	648	-0.968	-2.425	0.015
> 2 yrs	478	-0.00007	-0.058	0.95
PMF patients				
All follow-up	329	-0.087	-0.258	0.80
0–2 yrs	329	-0.414	-0.414	0.67
> 2 yrs	240	-0.009	-0.009	0.99

*Two-sided.

TABLE 6.—Cox regression analysis relating proportion of drug received to disease-free survival (time lag of 2 mos)

Time interval	No. of patients	Coefficient	Standardized coefficient	*P* value*
Placebo patients				
All follow-up	164	-0.269	-0.625	0.53
0-2 yrs	164	-0.331	-0.480	0.63
> 2 yrs	102	-0.719	-1.298	0.19
PF patients				
All follow-up	645	-0.171	-0.547	0.58
0-2 yrs	645	-0.342	-0.756	0.45
> 2 yrs	478	-0.019	-0.044	0.97
PMF patients				
All follow-up	324	0.097	0.275	0.78
0-2 yrs	324	0.230	0.399	0.70
> 2 yrs	235	0.00004	0.033	0.97

*Two-sided.

the time of treatment failure ($P = 0.02$) (table 5). For patients receiving PMF, no association between percent dose received and disease-free survival is evident. Similar evaluation of percent dose of placebo received suggests an association between dose and disease-free survival, with higher doses having a better result ($P = 0.05$). The strength of the association is almost the same for the period of therapy and following completion of therapy. When the last course of therapy prior to a treatment failure was disregarded in the analysis, the evidence for a dose-response relationship for PF was no longer significant ($P = 0.45$) (table 6). Similarly, when the last dose of placebo is disregarded, this association is also diminished for the period of time when patients are receiving placebo. The placebo findings indicate the need for caution in concluding a cause-effect relationship from observed associations between percent dose of chemotherapy and disease-free survival.

COMMENT

In view of the methodologic problems described, it is difficult to perceive how retrospective studies which purport to show a relationship between the amount of drug received and patient outcome can produce findings without statistical biases. Because such efforts are apt to have important biologic as well as clinical implications, they require critical evaluation prior to their unqualified acceptance. The difficulty, if not impossibility, of evaluating treatment efficacy after completion of a study by retrospectively delineating subgroups of patients according to amount of therapy received has also been demonstrated by findings from several clinical trials for diseases other than cancer. In one study evaluating the worth of tolbutamide therapy for diabetes (9) and in another assessing the efficacy of the lipid-influencing agent clofibrate for coronary heart disease (10), it was found that patients who took full or nearly full doses of those prolonged therapies fared better than did patients who were less compliant. Similarly, however, control patients who complied with the protocols did better than control patients who were noncompliant. In both studies the compliant control and treated patients did equally well. Patients who were noncompliant in either study had similarly poor results, indicating that noncompliers differ from compliers for reasons unrelated to the therapeutic effect. Thus, positive dose-response relationships must be interpreted with caution.

The present report demonstrates that it is difficult to relate total dose to results from initiation of therapy onward. It is not clear how the problem was overcome in studies where an attempt was made to correlate the amount of drug received and patient outcome (1,3). The methodology presented in the CMF study indicates that the drug dose received by patients with treatment failures was based on the number of courses given to the time of treatment failure (1). Utilizing a similar approach with our data (Method II), an opposite relationship between dose and response to that obtained in the CMF analyses was observed. The results in that study generally resemble the uniformly positive findings that we obtained using the first method for defining dose. The comparison of patients who received therapies for different durations (eg, six or 12 cycles of CMF) also presents methodologic difficulties, since percent dose received by patients failing during therapy will be related to the prescribed length of the therapy. The length of the therapy will affect the end results within dose groups for reasons unrelated to the amount of drug received, as demonstrated by us with placebo on Protocol B-05. The use of Method I (or an equivalent method) is implied in the comparison of six to 12 cycles of CMF, as well as by the total absence of treatment failures during the 1 year of therapy for patients receiving ≥ 85% of the intended dose of CMF. Hence, it is impossible for the group receiving Level I therapy for 1 year to have a poorer outcome during that year.

Findings from a study by the Southwest Oncology Co-

operative Group employing combination chemotherapy (CMFVP) versus melphalan also point to the dilemma. Results from that investigation (3) failed to indicate any clear relation between the percent of dose received and patient outcome. That comparison was subjected to the same biases which have been described for our Method I. Moreover, there is an additional problem in that the different regimens were administered for a different length of time. The amount of bias is thus different for the different therapies.

Another concern relates to the total confounding of age with the dose administered to older patients. Previous analyses of data have indicated that there is a differential response by age; younger women do better than the older women. If the treatment effect is age-related and drug dose is differentially administered according to age, then stratifying by age or dose accomplishes essentially the same result. Since there is a relationship to age, there will appear to be a relationship to dose. Statistical analysis cannot compensate for this type of total confounding.

This report is presented to emphasize that retrospective analyses, even when directed toward overcoming the many potential biases, must be regarded with caution. Results from them must be considered exploratory and not conclusive in nature. In any positive dose-response relationship, it is difficult, if not impossible, to distinguish cause from effect. In other words, do patients complying and receiving full doses do well, or do patients doing well comply and receive full doses? Prospective trials a priori designed to take into account the biases must then be carried out to obtain definitive findings.

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Comment on "The Methodologic Dilemma in Retrospectively Correlating the Amount of Chemotherapy Received in Adjuvant Therapy Protocols With Disease-Free Survival"[1]

Gianni Bonadonna* **and Pinuccia Valagussa**[2]

For many years, the chemotherapy of human neoplasms has been designed and executed on an almost purely empirical basis. In particular, drug dose and schedule have always been tailored to avoid severe and prolonged toxic effects, especially myelosuppression. To minimize the possibility of myelosuppression and to prevent regrowth of neoplastic cells which may occur when the interval between treatments is prolonged, dose reduction schedules have been designed to allow for continuous treatment with lower doses of myelosuppressive agents, even when moderate leukopenia and thrombocytopenia are present. The percent response related to the actual dose administered is usually not included in the detailed evaluation of treatment results. Even the first National Cancer Institute report on MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) chemotherapy for Hodgkin's disease (1) evaluated the amount of drugs delivered in each of the first six cycles as a function of bone marrow toxicity, and it was > 10 years until the dose level and timing were studied in relation to treatment outcome (2,3).

In our CMF (cyclophosphamide, methotrexate, and 5-FU) adjuvant program for breast cancer, the total relapse-free survival (RFS) proved to be superior in the chemotherapy-treated group compared to the control group (4), and results continue to be highly significant in the recent 8-year analysis (CMF, 46.5%; control, 33.5%; $P < 0.001$). Our retrospective evaluation published in 1981 (5) represented an attempt to find an explanation for why CMF appeared to exert only a transient efficacy in certain disease subsets. This analysis showed that RFS was also related to the dose level of drugs administered. Figure 1 shows that such a relationship persists up to 8 years from mastectomy and that in women who received < 85% of the optimal dose, RFS did not differ dramatically from that of the control group.

In a commentary appearing in this issue (6), Redmond et al, using four methods of analysis, failed to observe a similar correlation between the amount of chemotherapy administered and the 5-year RFS when adjuvant melphalan (P) was given alone or was combined with 5-FU (PF) and methotrexate (PMF). Actually, they noticed a correlation, but utilizing a methodology that we (and they) find inappropriate. In fact, when Method I is applied, patients with early failure are automatically excluded from Level I and are concentrated in the Level III group. It is perplexing that the National Surgical Adjuvant Breast and Bowel Project (NSABP) investigators were unable to find a positive correlation between dose level and RFS utilizing Method II, which is what we used in the analysis of our CMF data. They argue that "patients who failed early would be more likely to have received ≥ 85% of the protocol dose."

We believe that the main difficulty of the NSABP group in finding a reliable correlation between the amount of drug received and RFS is probably due to the fact that with all three regimens administered for 2 consecutive years, a high fraction of women showed treatment failure before adjuvant chemotherapy was completed (7). In contrast, relapse during CMF administration occurred in only 7.2%. At this point, we wish to reiterate that patients who failed during adjuvant chemotherapy with CMF were not automatically included in the group with a suboptimal dose, if they had received ≥ 85% of the projected dose of CMF at the time of treatment failure (8).

The remarkable difference in terms of early treatment failure between our three-drug combination and the three-drug regimens utilized by the NSABP could reflect the superior effectiveness of CMF compared to P, PF, and PMF. In fact, many research groups (4) have confirmed that administration of CMF at standard doses to women with clinically metastatic breast cancer resulted in complete plus partial remission rates ranging from 50% to 55%, while a low-dose regimen produced inferior results (table 1). In a randomized study (11), melphalan

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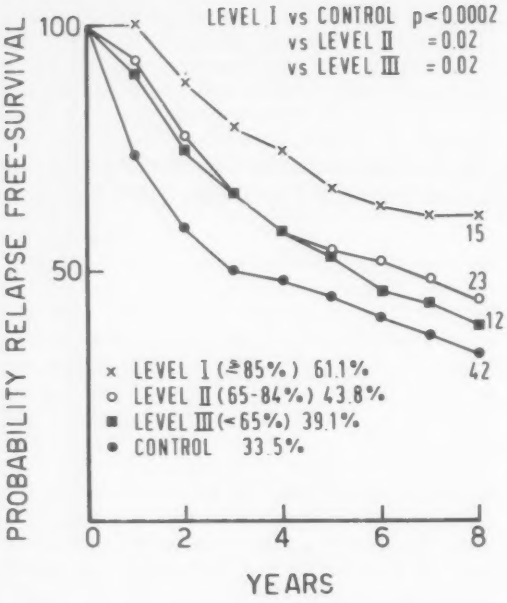

FIGURE 1.—CMF adjuvant program. Relation of RFS to dose level: 8-yr analysis.

alone produced a significantly lower response rate (20%) than CMF (53%). This difference in response rate has also been observed in advanced breast cancer by all investigators when CMF or CMFVP (cyclophosphamide, methotrexate, 5-FU, vincristine, and prednisone) or FCP (5-FU, cyclophosphamide, and prednisone) were randomly tested against melphalan in an adjuvant situation (12-14). To our knowledge, there is no published report demonstrating the efficacy of either PM or PMF in advanced breast cancer. Thus, in our opinion, one could expect to find a dose-response effect for a moderately chemosensitive tumor such as breast cancer only if the drug or drugs utilized in the regimen produce objective tumor response in at least 50% of the patients. For a response rate of lesser magnitude, it seems unlikely that one could detect a reliable correlation between dose level and response rate either in advanced disease or in an adjuvant situation.

In conclusion, Redmond et al are correct in stressing the difficulty (not the impossibility) of evaluating retrospectively the efficacy of drug levels administered and in recommending the utilization of a correct method of analysis. What is missing in their paper is the pharmacologic concept of drug treatment and its implications. Recent results from our Institute (15) as well as from other research groups (16-18) indicate that comparable, if not superimposable, results can be achieved in the adjuvant therapy of breast cancer with treatment of relative short duration utilizing the same multidrug regimen. This would confirm that tumor cell kill is not necessarily related to the total amount of drug received but to the intensity of treatment, ie, the peak level of the drugs administered in each course and delivered within the first few months of adjuvant treatment. This key pharmacologic concept has been confirmed in humans by retrospectively analyzing the mean rate of drug delivery during the first three cycles of MOPP for Hodgkin's disease (2), ie, in a tumor highly sensitive to effective chemotherapy. Therefore, in most patients, maximal tumor cytoreduction occurs within the first few months, when it is possible (and advisable) to deliver full or nearly full doses. This concept would explain why we were unable to find any important difference after either 12 or six cycles of adjuvant CMF, even when dose levels were evaluated (15). Prolonged treatment with the same regimen is not likely to improve the outcome and merely selects the overgrowth of specifically drug-resistant neoplastic cells. In such cases where prolonged administration of ineffective therapy fails to prevent a high fraction of relapses during treatment, the attempt to correlate the total amount of

TABLE 1.—Standard vs low-dose chemotherapy in advanced breast cancer

Group and treatment*	No. of patients	Response rate (%)†	Median duration of response (mos)	Ref No.
Milan				
AV	51	51	10.5	9
CMF (standard dose)	53	53	10.5	
Piedmont Oncology Association				
VAC	45	47	9	10
CMF (low dose)	44	16	6	

*AV = doxorubicin and vincristine; VAC = vincristine, doxorubicin, and cyclophosphamide.

†Complete plus partial.

drug delivered and the RFS will understandably raise difficulties in the interpretation of results.

Last but not least, we wish to re-emphasize that our retrospective analysis was not specifically aimed at evaluating compliance with protocol requirements, since a dose attenuation schedule for toxicity was indeed planned in our study protocol (5). Rather, the findings derived from our analysis strongly suggest that in an adjuvant setting, the appropriate drug administration (in terms of dosage, timing, sequence, route, and duration) of effective regimens is essential.

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Kaposi's Sarcoma: A New Staging Classification[1,2,3]

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Reports of an unusual form of Kaposi's sarcoma in young homosexual men in North America have demonstrated that previous classification systems for this disease are incomplete. We describe the clinical characteristics of 49 homosexual men with Kaposi's sarcoma and propose a new staging for the disease. There appear to be four clinically distinct forms of Kaposi's sarcoma: *stage I*—the more typical locally indolent lesions occurring predominantly in elderly males in North America and Europe; *stage II*—a locally invasive and aggressive form seen almost exclusively in equatorial Africa; *stage III*—a disseminated mucocutaneous form, often with lymph node involvement, seen primarily in African children and North American male homosexuals; and *stage IV*—a disseminated, mucocutaneous form with visceral involvement also seen in African children and North American male homosexuals. These stages are further subtyped as to the presence or absence of the systemic signs of unexplained fever and/or weight loss. Further longitudinal follow-up of these recently diagnosed cases will hopefully document that this proposed staging system correlates with survival data and is useful in the evaluation of treatment regimens for uniformly defined patient groups. [Cancer Treat Rep 67:531–534, 1983]

Until recently, Kaposi's sarcoma was considered a rare disease in North America and Europe, most commonly occurring in elderly males (1). It usually appears as violaceous macules, plaques, or nodules localized to a lower extremity. A more locally invasive and a disseminated form of Kaposi's sarcoma with lymphoreticular and visceral involvement has also been described in an endemic area of equatorial Africa (2). In Uganda, for example, Kaposi's sarcoma accounts for 9% of all malignant neoplasms in males (2).

The most widely used classification of Kaposi's sarcoma was published by Taylor et al (2) in 1971 (table 1). The four clinical types seemed to correlate well with the disease presentation as well as with the histopathology of the African cases.

Since 1979, > 100 North American patients with a disseminated type of Kaposi's sarcoma have been reported to the Centers for Disease Control (3). All have been homosexual males. Almost one-half of these cases have been seen at the New York University Medical Center. These patients all seem to have an aggressive, often lymphadenopathic form of Kaposi's sarcoma and thus would be grouped within one category of the Taylor classification. However, as patients were being evaluated it became obvious that certain characteristics not included in the Taylor classification may have prognostic and therapeutic importance and therefore should be considered in any stratification schema.

In this paper, we describe the patients seen at New York University and propose a comprehensive staging classification for Kaposi's sarcoma that more precisely subdivides the disseminated disease recently seen in male homosexuals. It is also suitable to describe the more "classic" forms of the disease seen predominantly in elderly males and in equatorial Africa.

METHODS

Twenty-seven cases of Kaposi's sarcoma in homosexual males have previously been described in detail

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TABLE 1.—Taylor classification of Kaposi's sarcoma (ref 2)

Clinical type	Behavior	Age group (yrs)	Bone involvement	Lymph node involvement	Predominant skin tumor
Nodular	Indolent	> 25	Rare	Rare	Nodules, plaques
Florid	Locally aggressive	> 25	Often	Rare	Fungating (exophytic)
Infiltrative	Locally aggressive	> 25	Always	Rare	Diffuse infiltration
Lymphadenopathic	Disseminated aggressive	< 25	Rare	Always	Nodules

(eight in ref 4, 19 in ref 5). The characteristics of those 27 patients and 22 more recent patients form the basis of this revision of the staging of Kaposi's sarcoma.

The most frequent presenting complaint was the appearance of one or more skin lesions. Numbers and locations of the lesions were noted, photographs were taken, and representative nodules or plaques were biopsied for pathologic confirmation of disease.

Lymphadenopathy was also common. Node biopsies were performed in most of the patients with accessible peripheral lymph nodes. Computerized tomographic (CT) scans of the abdomen and pelvis permitted evaluation of both the retroperitoneal and mesenteric lymph nodes as well as evaluation of spleen size.

Visceral involvement was documented as follows: gastrointestinal tract—endoscopy, colonoscopy, and gastrointestinal contrast studies; lung—bronchoscopy when the chest x-ray was abnormal; liver—radioisotope or CT scan; and bone—bone scan when the alkaline phosphatase was abnormal. Presence or absence of fever 100°F orally) and significant weight loss (≥ 10%) was also noted.

RESULTS

The sites of disease documented at the time of presentation for these Kaposi's sarcoma patients included skin, lymph nodes, spleen, liver, gastrointestinal tract, bone, and lung. Several different kinds of skin lesions were seen. They ranged from faint, pinkish-red macules or papules, only a few millimeters in size, to plaques or elevated nodules ranging in color from bluish-purple to red-brown and varying in size from 5 to 40 mm. Eight percent of the patients had no skin involvement whatsoever. Twenty-seven percent of the patients had fewer than five skin lesions at presentation. Sixty-three percent of the patients had innumerable skin lesions widely distributed over their skin surface area. One patient (2%) had a locally aggressive, exophytic lesion.

Sixty-one percent of the patients (30 of 49) had generalized lymphadenopathy at the time of first examination. Twenty-four of these 30 patients (80%) had lymph node biopsies, all of which confirmed the presence of Kaposi's sarcoma. The six patients with lymphadenopathy who did not undergo lymph node biopsy had other documentation of extracutaneous disease, predominantly in the gastrointestinal tract. Four patients had generalized lymphadenopathy, with no skin or demonstrable visceral organ involvement at the time of presentation. All four had biopsy-proven Kaposi's sarcoma in the lymph nodes. CT scans of the abdomen and pelvis usually showed evidence of retroperitoneal and/or mesenteric lymph node involvement in those patients with lymphadenopathy.

The spleen was enlarged to palpation and on CT or radioisotope scanning in five patients, two of whom underwent splenectomy documenting Kaposi's sarcoma. Therefore, splenomegaly in this setting probably represents splenic involvement. Similarly, hepatomegaly may signify disease within the liver. Biopsies were performed in three of five patients with hepatomegaly; all three demonstrated Kaposi's lesions.

In 45% of the patients studied (22 of 49), Kaposi's sarcoma lesions were found in one or more sites along the gastrointestinal tract. Almost all of these patients (21 of 22) had gastrointestinal tract lesions in association with generalized cutaneous disease. Fifty-five percent of these patients had only minimal gastrointestinal disease (arbitrarily defined as fewer than five lesions and < 2 cm in combined diameters) and appeared to manifest no increase in signs or symptoms beyond those seen in patients with generalized cutaneous lesions alone.

In three of the first eight reported cases of disseminated Kaposi's sarcoma, pulmonary disease was a cause of death. Since that time, three patients have had fever and reticulonodular infiltrates on initial chest x-ray. Bronchoscopy confirmed a diagnosis of *Pneumocystis carinii* pneumonia alone in one patient, *P. carinii* and Kaposi's sarcoma in the second, and Kaposi's sarcoma alone in the third.

Twenty-nine percent of the patients had either fever or weight loss when first seen. These data are summarized in table 2.

To account for this variation in disease presentation and to enable us to develop rational treatment strategies, we have classified patients as follows:

Stage

I—Cutaneous, locally indolent

II—Cutaneous, locally aggressive with or without regional lymph nodes

III—Generalized mucocutaneous and/or lymph node involvement *

IV—Visceral

* Generalized = more than upper or lower extremities alone; includes minimal gastrointestinal disease defined as < 5 lesions and < 2 cm in combined diameters.

Subtype

A—No systemic signs or symptoms

B—Systemic signs: weight loss (10%) or fever (> 100°F orally, unrelated to an identifiable source of infection lasting > 2 weeks)

Stage I represents the usual Kaposi's sarcoma type seen in elderly patients; stage II represents the African-type Kaposi's sarcoma when locally invasive; and stages III–IV stratify the disseminated Kaposi's sarcoma described in homosexual males and in African children. Each stage is further subtyped as to the absence or presence of fever (unrelated to an identifiable source of infection) and/or weight loss (≥ 10%): subtypes A and B, respectively.

Six patients (12%) had stage I disease, one (2%) had stage II disease, 29 (59%) had stage III disease, and 13 (27%) had stage IV disease. Seven of the 29 stage III patients (24%) had B symptoms, whereas eight of the 13 stage IV patients (62%) had B symptoms (table 3).

DISCUSSION

In the first case reports of the disseminated form of Kaposi's sarcoma in North American male homosexuals, five of the eight patients died from either progressive disease or infection (4). More cases have since appeared with a much broader spectrum of disease presentation. Other authors have also noted a generalized form of the disease that occurs in approximately 5% of the African patients (1,2). This type of Kaposi's sarcoma usually afflicts children and young adults, involves lymph nodes and visceral organs, and clinically behaves very much like the rapidly progressive disease in the male homosexuals described above. However, the currently used classification system of Taylor et al does not provide for sufficient stratification of such patients. Some patients, with disease as far advanced as in the original cases, require aggressive treatment. Those patients who are less ill may benefit from less toxic therapy. The staging system described herein was created to guide these therapeutic decisions. It is also useful in analyzing results of any therapeutic trial by assuring that similar patient groups are studied.

TABLE 2.—Sites of disease and systemic signs at presentation in 49 patients with Kaposi's sarcoma

	No. of patients (% of total group)
Skin lesions	
Localized	13 (27)
Generalized	31 (63)
Locally aggressive	1 (2)
None	4 (8)
Lymph node involvement	
Localized	—
Generalized	30 (61)
Spleen	5 (10)
None	19 (39)
Visceral involvement	
Bone	1 (2)
Lung	5 (10)
Liver	5 (10)
Gastrointestinal tract	22 (45)
Minimal	12 (25)
Extensive	10 (20)
Systemic signs*	
Fever and weight loss	9 (18)
Fever only	4 (8)
Weight loss only	1 (2)
Total	14 (29)

*Unexplained fever > 100°F (orally) and ≥ 10% weight loss.

TABLE 3.—Staging summary of 49 patients*

Stage	A	B	Total
I	6	—	6
II	1	—	1
III	22	7	29
IV	5	8	13

*Patients with stage IV disease are significantly more likely to have B symptoms than stage III patients ($X^2 = 3.96$, $P < 0.05$).

Initially, we considered using a T-N-M classification, as used for most malignancies. However, Kaposi's sarcoma, like the malignant lymphomas, appears to be a multicentric disease with more extensive presentations representing more aggressive disease rather than metastatic spread. Early disease is usually confined to the skin and to the extremities alone (stage I). This is the common variety of Kaposi's sarcoma seen in elderly patients, usually males with local, indolent, cutaneous lesions. In the "African" form these extremity lesions become exophytic or locally invasive to the underlying bone (stage II). In the current epidemic of Kaposi's sarcoma most patients present with more generalized disease (42 of the 49 patients in this series). Generalized has been defined as more than upper or lower extremities with cutaneous disease alone to distinguish it from the local lesions of stages I–II.

One advantage of the proposed classification schema is the subdivision of these patients with generalized skin lesions into two groups: adenopathy with or without minimal gastrointestinal disease (stage III), and visceral disease (stage IV). Patients with minimal gastrointestinal tract involvement (arbitrarily defined as five lesions and 2 cm in combined diameters) appear to do no worse than patients without gastrointestinal tract involvement and therefore are included in stage III. Stage III also includes involvement of the spleen or more than one region of lymph nodes. Patients with generalized Kaposi's sarcoma with lymph node involvement usually have extensive lymphadenopathy. Splenomegaly, for the purposes of this clinical staging system, is assumed to represent splenic involvement. Stage IV includes all patients with visceral disease other than minimal gastrointestinal tract

involvement. The most common sites other than the gastrointestinal tract are liver and lung.

Each stage is subtyped as A or B depending on the absence or presence, respectively, of the systemic signs of unexplained fever and/or 10% weight loss. Patients with stage IV disease have a significantly greater incidence of the B symptoms than patients with stage III disease ($P = 0.05$).

Thus, both Kaposi's sarcoma and its proposed staging system bear many similarities to lymphoreticular neoplasms. There is an increased association of Kaposi's sarcoma with lymphoreticular neoplasms (6); each may be related to a viral etiology (cytomegalovirus and Epstein-Barr virus) (7), and both occur in immunosuppressed patients (8). There is an increased incidence of Kaposi's sarcoma in patients who have undergone renal transplants (9), patients receiving corticosteroid therapy (10), and the recently described group of male homosexuals with *P. carinii* pneumonia who had documented cellular immune dysfunction (11–13). Thus, it seems reasonable that the staging system for Kaposi's sarcoma should resemble that for malignant lymphomas.

Most of the patients identified when the epidemic was first described had late disease. More recently, patients have presented with earlier stages; 45% of the patients (22 of 49) in this series had stage IIIA disease. This probably represents an increased awareness of the disease both in the homosexual and the medical communities. However, should stage IIIA remain the predominant subtype in future cases, it may become necessary to revise this classification schema.

We are currently using this new system for the staging of Kaposi's sarcoma to identify patients requiring early aggressive combination chemotherapy (stage IV and patients with B symptoms) and those who can be started on single-agent trials (stages II–III), as well as those who can be treated with local treatments such as radiation therapy (stage I). Further longitudinal follow-up of these recently diagnosed cases will hopefully document that this proposed staging system correlates with survival data and is useful in evaluating treatment regimens for uniformly defined patient groups.

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Integration of Safe Initial Combination Chemotherapy (Without Cisplatin) With a High Response Rate and Local Therapy for Untreated Stage III and IV Epidermoid Cancer of the Head and Neck: 5-Year Survival Data[1,2]

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This prospective study has established the effectiveness and safety of two courses of a combination of vincristine, bleomycin, methotrexate, 5-FU, and hydrocortisone with a folinic acid "rescue" given over 24 hours on Days 1 and 14 prior to surgery and/or radiotherapy in 93 patients with advanced (stage III and IV) untreated epidermoid cancer of the head and neck. Of 91 patients evaluated on Day 28 for chemotherapy response, 58 (64%) had a partial remission and 33 (36%) were classified as nonresponders, although 12 had a minimal response of 20%–30%. After completion of local therapy, 73% of the chemotherapy responders achieved complete remission compared with only 48% of the nonresponders ($P = 0.005$). Forty-seven percent of the patients achieving complete remission were alive at 5 years compared with only 4% of those with residual disease after local therapy. Response to chemotherapy is a good prognostic sign. Side effects were minimal and there was 100% patient compliance. [Cancer Treat Rep 67:535–539, 1983]

The role of chemotherapy in the treatment of advanced stage III and IV epidermoid carcinoma of the head and neck is being reconsidered. Originally, chemotherapy in head and neck cancer was aimed at palliating inoperable or advanced recurrent disease. However, several studies have shown that response to chemotherapy is significantly reduced in previously irradiated patients (1–4). This had led to studies which either use chemotherapy as initial therapy prior to local therapy or integrate chemotherapy simultaneously with radiotherapy. O'Connor et al (5), adopting this latter approach, have shown increased survival compared with a historical control group, but this was accompanied by some severe side effects often necessitating interruptions and delay of the planned radiotherapy. We used the alternative approach and gave a combination of vincristine, bleomycin, methotrexate, and 5-FU (Schedule A) as initial therapy, with a high response rate of 75% and minimal toxicity (2).

Details of the theoretic rationale for designing Schedule A in 1973 have been described previously (6,7). The drugs selected had shown some activity as single agents in head and neck cancer. Briefly, drugs were administered over approximately 24 hours based on the experimental studies of Bruce and his colleagues, who showed increased selective toxicity of anticancer drugs against malignant stem cells in tumor-bearing mice under these conditions. Cell kinetic studies also provided some evidence that the sequencing of drugs within this 24-hour time period might be important in increasing their selectivity against tumors. 5-FU was given after methotrexate because of some experimental evidence that synergism between these two drugs might be achieved using this sequence. Hydrocortisone was included in the regimen in an attempt to prevent the immediate anaphylactic reactions to bleomycin and to try to prevent long-term side effects, such as pulmonary fibrosis.

This is a report of a study to determine feasibility, effectiveness, and toxicity in the adjuvant use of Schedule A chemotherapy in advanced previously untreated stage III and IV epidermoid cancers of the head and neck. Re-

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gretably, the radiation therapists to whom these patients are initially referred are not prepared to conduct a randomized, prospective, controlled trial. However, since preliminary data (8) indicate that primary tumor site may be a significant prognostic factor, we have continued to accrue patients into this study. We now report, for the first time, 5-year survival rates for patients treated in this way with initial chemotherapy.

MATERIALS AND METHODS

Ninety-three patients with advanced, untreated, histologically proven epidermoid carcinoma of the head and neck were eligible for this study. Prior therapy of any kind, or the presence of metastases beyond the regional lymph nodes, made the patients ineligible. Patients with a history of pulmonary disease in whom a pulmonary diffusion defect was thought to be present, as shown by a low dCO or radiologic evidence of pulmonary fibrosis, were excluded from this study (two patients), as were those with markedly impaired renal function, as judged by a creatinine clearance < 60 ml/minute (one patient). All patients were ambulatory at the time of their first treatment. Fifty-nine patients (63%) had stage III disease and 34 (37%) had stage IV disease, according to the International Union Against Cancer pretreatment clinical classification for head and neck tumors (1978 edition). Seventy-one patients were male and 22 were female, with a median age of 61 years (range, 15–80). Details of the primary tumor site are provided in the Results section. A complete morphologic study has been made of only 69 of the 93 epidermoid tumors. Of these tumors, 18% were well-differentiated, 28% were moderately differentiated, 42% were poorly differentiated, and 12% were undifferentiated. A full review of the histologic classification is now underway and will be reported elsewhere. Patients were scheduled to receive two courses of Schedule A combination chemotherapy on Days 1 and 14. The drugs were given as follows: vincristine, 2 mg iv at 0 hour; bleomycin, 60 mg as a 6-hour infusion from 12 to 18 hours; methotrexate, 100 mg iv at 12, 15, and 18 hours; 5-FU, 500 mg iv at 18 hours; hydrocortisone, 500 mg iv at 12 and 18 hours; and folinic acid, 15 mg orally at 26, 32, 38, and 44 hours. In the kinetic rationale that we used, based on the Bruce classification of antitumor drugs, flat doses are appropriate for vincristine and methotrexate administered over 24 hours since, provided a tumoricidal level is achieved, there is no increase in tumor cell kill with increasing dose. Bleomycin is not included in that classification, since it is not myelosuppressive in this dose range, and a flat dose was used. 5-FU, originally scheduled as 400 mg/m^2, almost invariably came out to the nearest 10 as 500 mg. The size distribution was not wide enough in this group of patients to utilize the per meter squared concept for 5-FU. On Day 28, patients were assessed for chemotherapy response and "curative" local therapy was initiated. Nearly all patients were referred for radiotherapy as primary treatment and surgery was subsequently integrated if response to radiotherapy appeared unsatisfactory. Surgery alone was chosen only if there was a chance of surgical cure, or where radiotherapy was thought to be unlikely to help, eg, in necrotic postcricoid carcinomas. In general, the policy was to irradiate to 4000 rads (40 Gy) and assess. Supraclavicular fields were routinely included if neck nodes were present. Implant therapy supplemented external-beam radiation therapy in 1% of the patients. Patients not responding to this radiation therapy were then considered for surgery. The object of surgery was always complete resection. The type of radiation used was usually tele-cobalt 60, external-beam, between 70 and 100-cm source to skin distance, but occasionally (ie, in postcricoid carcinomas) the linear accelerator (4–8 mV) was used. If no surgery was added, approximately 65 Gy was given over 6–7 weeks as 10 Gy over 5 days/week. Following completion of local therapy, the patients were monitored for signs of disease recurrence but received no further chemotherapy in the absence of relapse. Sixty-seven percent of the patients received radiotherapy only, 29% were treated with radiotherapy and salvage surgery, and 4% had surgery alone. Definition of response is as follows: *complete remission (CR)*—the disappearance of all clinically measurable lesions; *partial remission (PR)*—a reduction of ≥ 50% in the product of two perpendicular diameters of all measurable lesions in the absence of any new lesion(s); and *minimal response*—a > 20% but < 50% reduction in the product of two perpendicular diameters of all measurable lesions in the absence of any new lesion(s).

Response rates were compared using the chi-squared test. Survival was calculated by a life-table method and compared using the log-rank test.

The following medical precautions were taken for all patients.

(*a*) No treatment cycles were repeated unless the peripheral wbc and platelet counts had returned to their original level.

(*b*) Patients with impaired renal function receiving methotrexate had an extended folinic acid rescue: if the creatinine clearance was > 90 ml/minute the protocol was given as stated, or if the creatinine clearance was between 60 and 90 ml/minute 12 doses of folinic acid (15 mg) were given (patients with a clearance < 60 ml/minute were excluded from the study).

(*c*) All patients were adequately hydrated and passed at least 2 L of urine during the 24 hours of drug treatment.

There were weekly checks for specific symptoms, physical examination, weight measurements, liver function tests, BUN, electrolytes, and peripheral blood cell counts in order to assess drug-induced toxic effects.

RESULTS

Of the 93 patients entered into this study, two courses of Schedule A chemotherapy were administered without any change of dosage or timing to 92 patients, as planned, and one patient received only one course. Ninety-one patients were assessed for response to chemotherapy. Reasons for nonassessment were: one patient, no initial measurements recorded; and one patient, death due to protocol violation (*see* later). Fifty-eight patients (64%) responded (at least a PR) to chemotherapy and 33 (36%) were classified as nonresponders, although 12 (13%) had a minimal response. Sixty-seven percent of the patients with stage III disease responded to chemotherapy compared with 56% of those with stage IV disease (not significantly different). The CR rate to chemotherapy alone in this series was only 6%, *but* this is misleading since radiation therapy was always started on Day 28 even in lesions that were continuing to respond. Therefore, it seems likely that if radiotherapy had been delayed and chemotherapy had been continued, far more would have been achieved by chemotherapy alone.

Preliminary data suggest that response was not significantly influenced by histologic grade. Table 1 shows that response was not influenced by sex, but patients with oral cavity lesions were more likely to respond to chemotherapy than those with tumors at all other sites ($P = 0.025$). Significantly more responses were seen in younger patients, the median age for chemotherapy responders being 56 years and for nonresponders, 63 years, with $P = 0.049$ according to the Kolmogorov-Smirnov test (9).

There was 100% patient compliance, and toxicity to Schedule A chemotherapy was minimal provided that standard precautions were observed. Fifty-one patients (55%) reported no side effects. Details of the side effects in the remaining 42 patients are shown in table 2. The one death occurred following a protocol violation since the patient had known impairment of renal function and was not given a prolonged folinic acid rescue as described in the precautions listed previously.

The overall CR rate after radiation therapy and/or surgery was 64%. Patients who responded to initial chemotherapy achieved a 73% CR rate after local therapy. This contrasted with only 48% for patients who failed to respond to chemotherapy achieving a CR. This difference is statistically significant ($P = 0.005$). Therefore, a response to initial chemotherapy significantly increases the likelihood of successful local treatment.

These patients were treated between January 1975 and December 1979, and the mean follow-up time was 48 months. The 5-year actuarial survival rate was 36% for the overall group. Chemotherapy responders have a longer median duration of survival than nonresponders (20.4 vs 12.3 months; $P = 0.049$). However, the most significant finding was that patients who achieved a CR after local therapy lived significantly longer ($P < 0.0000001$) than those with residual disease; the median duration of survival was 52.5 and 7.2 months, respectively. At 5 years (fig 1), 47% of the patients achieving a CR are alive compared with only 4% of those not achieving a CR. Since chemotherapy responders are significantly more likely to achieve a CR than nonresponders, response to Schedule A chemotherapy is a good prognostic sign.

DISCUSSION

This study confirms earlier reports (1,2) showing that

TABLE 1.—Prognostic factors in 91 patients assessed for chemotherapy response

Characteristic	No. of chemotherapy responders	No. of chemotherapy nonresponders
Sex		
Male	46 (65%)	25
Female	12 (60%)	8
Age (yrs)		
< 20	3	0
20-29	1	0
30-39	3	0
40-49	11	1
50-59	15	8
60-69	17	20
> 70	8	4
Site of disease		
Oral cavity	17 (85%)	3
Oropharynx	11 (55%)	9
Nasopharynx	5 (83%)	1
Hypopharynx	7 (54%)	6
Larynx	17 (55%)	14
Unknown primary	1	0

TABLE 2.—Side effects from 185 courses of Schedule A chemotherapy*

Side effect	No. of cycles
Myelosuppression	
Wbc count nadir < 2500/mm^3	1
Platelet count nadir < 100,000/mm^3	0
Mucositis (mild, no intubation required)	8 (4%)
Nephrotoxicity	0
Peripheral neuropathy (mild)	7 (4%)
Pulmonary (chest pains)	1
Cardiovascular (atrial fibrillation)	1
Skin rash/pigmentation (mild)	9 (15%)
Anorexia, nausea, vomiting (mild)	12 (6%)
Alopecia (partial)	6 (3%)
Malaise or lethargy	9 (5%)
Death from treatment (protocol violation)	1
Other	12 (6%)†

*No. of patients *without* side effects, 51 (55%); No. of patients *with* some side effects, 42 (45%).

†Includes 2 patients with headaches, 2 with constipation, 1 with indigestion, 1 with exacerbation of peripheral vascular disease, 1 with earache, 1 with pyrexia, 1 with diarrhea, 1 with muscular ache in inguinal region, 1 with ankle edema, and 1 with dizziness.

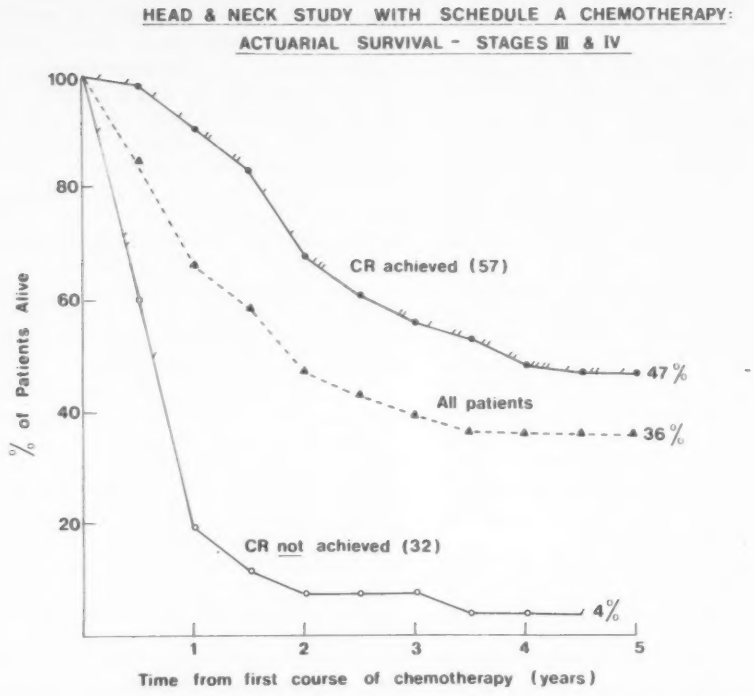

FIGURE 1.—Actuarial survival rates for all patients comparing those who achieved a CR after initial chemotherapy with Schedule A followed by "curative" local therapy with those with residual disease at assessment. Marks represent censored observations on patients still alive or lost to follow-up.

Schedule A chemotherapy has a consistently high response rate in stage III and IV head and neck tumors when used as initial treatment. This compares favorably with other current protocols (10-12). The minimal toxicity associated with this method of administering antitumor agents over 24 hours, which we have reported for treating other "solid" tumors (13), is again confirmed here. The safety of this schedule has been confirmed by several other groups (14-16). These studies, therefore, all contrast with the moderate toxicity reported by Raafat and Oster (17) using this schedule in 15 patients. These authors, however, failed to state whether the routine medical precautions were strictly observed.

It may be of interest that the 5-year survival rate for all patients was 36%, which compares favorably both with the 22% rate for a historical control group treated with local therapy alone at the Royal Marsden Hospital prior to 1975 and the 4-year rate of 24.5% for a radiotherapy control group quoted by O'Connor et al (5). However, the observation that a response to two initial courses of Schedule A chemotherapy increased the likelihood of CR following local treatment is of particular importance since we show here that, after a 5-year follow-up, 47% of the patients achieving a CR remain alive and well. This is significantly more ($P < 0.0000001$) than those failing to achieve a CR after local therapy.

There is a major need for randomized, prospective, controlled clinical studies using combination chemotherapy prior to local therapy to establish whether improved overall survival can be achieved. The safety and efficacy of this Schedule A protocol make it an attractive candidate for such a trial. The results would also establish whether the very frequent inclusion of cisplatin and its associated toxicity in initial chemotherapy regimens is justified.

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Cytotoxic Activity of 5′-Deoxy-5-Fluorouridine in Cultured Human Tumors[1,2]

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The cytotoxic activity of 5′-deoxy-5-fluorouridine (5′-dFUrd) was established in six cultured human tumor lines: 47-DN and MCF-7 breast carcinomas, MG-63 osteosarcoma, HCT-8 colon carcinoma, Colo-357 pancreatic carcinoma, and HL-60 promyelocytic leukemia. Cells were exposed to a wide range of 5′-dFUrd concentrations (from 0.1 μM to 1.0 mM) for 3, 6, or 24 hrs, and then cloned using standard in vitro clonogenic assays. 5′-dFUrd exhibited its best activity in the 47-DN and MCF-7 breast cell lines and in the MG-63 osteosarcoma line (3-hr LD50 values of 32, 34, and 38 μM, respectively). Less activity was observed in the HCT-8 colon (LD50 = 195 μM) and Colo-357 pancreatic (LD50 = 155 μM) tumor lines, and very poor activity was noted in the HL-60 leukemia cell line (LD50 = 465 μM). The metabolism of 5′-dFUrd to 5-FU (FUra) and FUra-nucleotides was determined and found to directly correlate with the potency of 5′-dFUrd in these cell lines. These results suggest that: (*a*) there is a marked variation in sensitivity of human cancer cells of different tissue origin to 5′-dFUrd, (*b*) there is a direct relationship between the sensitivity of human cells to 5′-dFUrd and the ability of the cell to metabolize 5′-dFUrd to FUra, and (*c*) increasing exposure period of cells to 5′-dFUrd did not markedly alter 5′-dFUrd potency in all human cancer cells examined, with the exception of the 47-DN breast cancer cells. [Cancer Treat Rep 67:541–545, 1983]

5′-Deoxy-5-fluorouridine (5′dFUrd) is a new synthetic fluoropyrimidine nucleoside of interest because of its superior therapeutic activity, compared to 5-FU (FUra) or other fluoropyrimidines, as an anticancer agent in rodents (1–3). 5′-dFUrd cytotoxic activity is dependent upon its cleavage to FUra (1,4,5). The basis for its improved therapeutic activity appears related to the selective activation of 5′-dFUrd by sensitive tumor cells compared to normal bone marrow stem cells (6).

While a variety of studies have been completed with 5′-dFUrd in rodent tumors, the sensitivity of human cancers has not been previously demonstrated. The present study was initiated to characterize the cytotoxicity of 5′-dFUrd in several types of cultured human cancers of different tissue etiologies. The relationship between cytotoxic potency, as measured using in vitro clonogenic assays, and the ability of the cell to metabolize 5′-dFUrd was also established.

MATERIALS AND METHODS

Drugs.—5′-dFUrd was supplied by Dr. A. E. Scott of Hoffmann-LaRoche, Inc, Nutley, NJ. [^{3}H]5′-dFUrd was purchased from Moravek Biochemicals, City of Industry, CA. All chemicals used in tissue culture were obtained from Gibco Laboratories, Grand Island, NY.

Human cell lines.—HL-60 cells (a human acute progranulocytic leukemia cell line) were maintained as a suspension culture in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, penicillin (100 IU/ml), and streptomycin (100 μg/ml). The doubling time (DT) was approximately 28 hrs. The solid human tumor cell lines [47-DN breast carcinoma (DT = 42 hrs), MCF-7 breast carcinoma (DT = 32 hrs), HCT-8 colon adenocarcinoma (DT = 18 hrs), Colo-357 pancreatic carcinoma (DT = 27 hrs), and MG-63 osteosarcoma (DT = 21 hrs)] were

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maintained as continuously growing monolayers. The 47-DN and MCF-7 breast cells were grown in RPMI-1640 medium supplemented as described above as well as with 1 mM estradiol and 2 IU/ml of insulin. The other cell lines were grown under similar conditions but without the insulin and estradiol. All cell lines were kept in a 5% CO_2-humidified incubator at 37°C.

Bilayer-agar cloning assay.—HL-60 leukemia cells and MG-63 osteosarcoma cells were cloned using bilayer-agar methodology similar to that reported previously (7). Cells were suspended for 3, 6, or 24 hrs in McCoy's medium either with or without drug and incubated at 37°C. Following this exposure period, the cells were centrifuged, washed in drug-free medium, and resuspended in 0.3% Bacto-agar in McCoy's 5A medium supplemented with 20% FCS. One milliliter of this mixture (containing 1000 cells for HL-60 or MG-63 tumor) was pipetted onto a 1-ml feeder layer consisting of 0.5% Bacto-agar in McCoy's 5A medium with 20% FCS. GCT-conditioned medium (Gibco) was added to cover the surface of the culture plate. Cultures were incubated at 37°C in 5% CO_2 in a humidified atmosphere with no additional feeding. Colony formation was quantitated visually with a microscope at × 100, 9–11 days after plating. This procedure allows for an approximately 25% cloning efficiency.

Monolayer cloning assay.—Clonogenicity of the solid human cancer cell lines 47-DN, MCF-7, HCT-8, and Colo-357 was examined using the monolayer cloning methodology as previously reported (8). Single-cell suspensions of cells were prepared from stock flasks by trypsinization and vigorous pipetting, and 12,000 cells (quantitated using a Model ZBI Coulter counter, Coulter Electronics, Inc, Hialeah, FL) were evenly seeded into 25-cm^2 tissue-culture flasks. Following a 24-hr period to allow cells to attach, the cells were treated with drugs for 3 hrs, washed, and then reincubated for 4 5 days in drug-free medium. When untreated cultures exceeded a median clonal diameter ⩾ 0.25 mm, the medium was decanted, the flasks were gently rinsed with phosphate-buffered saline and immediately stained with a methanol-crystal violet (25% solution); colonies were then counted on an automatic colony counter (Biotran II, New Brunswick Scientific Co, Inc, Edison, NJ).

Metabolism studies.—Approximately 3×10^7 cells were exposed for 60 mins to 0.1 mM [^{3}H]-5′-dFUrd (specific activity, 10 μCi/μmol). Cells were then immediately isolated by rapid centrifugation and disrupted by the addition of 0.2 N $HClO_4$. The acid-soluble extract was isolated and buffered to pH 6.0, and the acid-soluble fluoropyrimidines were characterized by high-performance liquid chromatography (HPLC). An Altex-Model 110-A liquid chromatograph was used for all fluoropyrimidine separations. Samples were separated on a 5-μm (30 × 0.4-cm) Ultrasphere-IP C-18 reverse-phase column (Rainin Instrument Co, Woburn, MA). Samples were eluted with: Buffer A (30 mM potassium phosphate, and 2.5 mM tetrabutylammoniumhydrogen sulfate, pH 6.5) and Buffer B (50% methanol and 50% Buffer A). Gradient conditions were as follows: 0–20 mins, 100% Buffer A; 20–60 mins, linear gradient to 50% Buffer B; and 60–80 mins, linear gradient to 100% Buffer B. Column eluate was collected in 1-ml fractions, and radioactivity was measured in a Beckmann LS-7000 liquid scintillation counter. This methodology allows for resolution of FUrd (7 mins), FUrd (12 mins), FdUrd (16 mins), FUMP (20 mins), FdUMP (31 mins), 5′-dFUrd (36 mins), FUDP (45 mins), and FUTP (61 mins).

RESULTS

Cytotoxicity of 5′-dFUrd in Human Tumor Cells

The cultured human tumor cells 47-DN, MCF-7, HCT-8, Colo-357, MG-63, and HL-60 were exposed to concentrations of 5′-dFUrd from 1.0 mM to 0.1 μM for 3, 6, or 24 hrs and then cloned as described in the Materials and Methods section. The cytotoxicity studies were completed in medium supplemented with either FBS or dialyzed FBS since this has been found to affect the sensitivity of cells to fluoropyrimidines (8,9). The LD50 values (calculated from the toxicity curves) for each cell line (grown in either FBS or dialyzed FBS) at the different exposure periods are listed in table 1. 5′-dFUrd exhibited its best activity against the two breast carcinomas, 47-DN and MCF-7, and then the MG-63 osteosarcoma. 5′-dFUrd was much less active in the HCT-8 colon and Colo-357 pancreatic tumor cell lines and exhibited extremely poor cytotoxic activity in HL-60 promyelocytic leukemia. This pattern was maintained regardless of whether cells were grown in FBS or dialyzed FBS. However, there were some differences in sensitivity depending on the type of serum used. The LD50 for a 3-hr exposure in 47-DN breast and Colo-357 pancreatic cells dropped 47% and 37%, respectively, when cells were grown in dialyzed FBS. Changes with the other cell lines were not notable.

The effect of exposure time on the activity of 5′-dFUrd is also shown in table 1. Some increase in cytotoxic activity occurred with an increased exposure period in each tumor cell line; however, the degree of change varied among them. The cell line most sensitive to an increased exposure period was the 47-DN breast carcinoma. This cell line appeared to respond differently to 5′-dFUrd than did the other cell lines, since only these cells exhibited a marked increase in sensitivity to 5′-dFUrd with both length of exposure and dialyzed FBS. This suggests that 5′-dFUrd may have an additional mechanism of cytotoxicity in these cells that is less prominent in the other human cell lines.

TABLE 1.—Cytotoxicity of 5′-dFUrd in human tumor cells

Cell type	LD50 (μM)* at— 3 hrs	6 hrs	24 hrs	% increase in activity†
Breast				
47-DN	32 (36)‡	25 (18)	11 (4.5)	66 (83)‡
MCF-7	34 (31)	32 (30)	29 (26)	15 (16)
Gastrointestinal				
HCT-8	195 (185)	175 (175)	160 (153)	18 (17)
Colo-357	155 (98)	142 (92)	136 (82)	22 (16)
Osteosarcoma				
MG-63	38 (35)	—	32 (30)	16 (14)
Leukemia				
HL-60	465 (476)	360 (368)	356 (360)	25 (24)

*Determined in vitro in clonogenic assays at exposure times of 3, 6, and 24 hrs.
†% increase in activity = 100 - [(LD50 (24 hrs) - LD50 (3hrs) × 100].
‡LD50 and % increase in activity determined from cells grown with dialyzed FBS are shown in parentheses.

Metabolism of 5′-dFUrd

To determine the ability of the cells to metabolize 5′-dFUrd, cells of each tumor line were exposed to 0.1 mM [^{3}H]5′-dFUrd for 60 mins, and the intracellular fluoropyrimidines were characterized by HPLC analysis. Table 2 illustrates the results obtained. Only FUra and FUra-ribonucleotide were identified as 5′-dFUrd metabolites. The MCF-7 and MG-63 cells exhibited the highest rates of metabolism of 5′-dFUrd, and these rates were much higher than those observed in the other cell lines. HL-60 leukemia cells were the least able to metabolize 5′-dFUrd to FUra and FUra-ribonucleotides. The incorporation of FUra into RNA of each cell line was also determined following a 3-hr exposure to 0.1 mM [^{3}H]5′-dFUrd. The results correlated with the metabolism study (pmols of FUra/μg of RNA): MCF-7, 1.08; MG-63, 0.91; 47-DN, 0.53; HCT-8, 0.48; Colo-357, 0.68; and HL-60, 0.07. The incorporation of FUra into RNA was the same regardless of whether cells were grown in dialyzed or regular FBS.

DISCUSSION

Cytotoxicity resulting from exposure of cells to 5′-dFUrd has been reported in murine tumors to be dependent on the enzymatic cleavage of 5′-dFUrd to FUra via a nucleoside phosphorylase (1,5). FUra, once formed, is then converted to the active nucleotides FUTP and FdUMP. FUTP can substitute for UTP in RNA biosynthesis, and this can result in a disruption of RNA function. FdUMP is a potent inhibitor of thymidylate synthetase activity, which can lead to a depletion of cellular thymidylate and a suppression of DNA synthesis. Previous investigations in mouse tumors have suggested that 5′-dFUrd cytotoxic activity is greatest through an inhibition of thymidylate synthetase activity, although at higher concentrations another mechanism (presumably mediated through the incorporation of FUra into RNA) did contribute to cytotoxicity (4,5). Both effects were dependent, however, on the breakdown of 5′-dFUrd to FUra. Further evidence illustrating the relationship between 5′-dFUrd

TABLE 2.—Metabolism of 5′-dFUrd in human tumor cells*

Cell line	% intracellular fluoropyrimidine 5′-dFUrd	FUra	Fluoronucleotide	% metabolism†
47-DN	92	5	3	8.2
MCF-7	81	12	6	18.5
HCT-8	94	4	2	6.2
MG-63	83	13	4	17.0
Colo-357	90	7	3	9.7
HL-60	99	0.96	0.14	1.1

*Cells were exposed to 0.1 mM [^{3}H]5′-dFUrd for 60 mins, and the intracellular acid-soluble metabolites characterized by HPLC as described in the Materials and Methods section.
†% conversion = 100 - [(5′-dFUrd cpm ÷ total cpm) × 100].

cytotoxicity and the cells' ability to metabolize 5'-dFUrd was the demonstration that the L1210 murine leukemia was resistant to 5'-dFUrd but not to FUra and that these cells lacked nucleoside phosphorylase activity (3). The absence of phosphorylase activity in bone marrow stem cells has also been suggested to represent the basis for the low level of hematopoietic toxicity resulting from 5'-dFUrd (6).

Previous cytotoxicity studies in other cultured human tumor lines have indicated that the mechanism of FUra cytotoxicity, regardless of exposure time, was unrelated to an inhibition of thymidylate synthetase activity (10). Sensitivity was also found to be more related to the concentration of drug than the exposure time. The results presented here with 5'-dFUrd indicate that similar patterns occur in the MCF-7, Colo-357, HCT-8, HL-60, and MG-63 human tumor lines. Additionally, the incorporation of FUra into RNA in these cell lines correlated with resulting toxicity. These results suggest that, for these cell lines, toxicity from 5'-dFUrd may be primarily RNA-directed. It is of interest that 5'-dFUrd may act differently in the 47-DN breast cells. These cells were the only line exhibiting a high degree of sensitivity to the exposure time, with longer periods of exposure to 5'-dFUrd resulting in increased cytotoxicity. Additionally, 47-DN cells were much more sensitive when grown in medium containing dialyzed FBS than when grown in medium containing FBS. Previous studies have indicated that FBS can contain levels of thymidine or hypoxanthine that can reduce the cytotoxic activity of fluoropyrimidines (8,9) which resulted in an apparent loss of DNA-directed toxicity. As a result, a mechanism of 5'-dFUrd activity in 47-DN cells may be mediated by an inhibition of thymidylate synthetase activity. As was observed above, this mechanism of action has previously been shown to result in the greatest cytotoxicity for 5'-dFUrd. Further evidence was that FUra incorporation into RNA was no different in these cells whether grown in either dialyzed or regular FBS, although toxicity varied greatly in 47-DN cells.

These studies establish in human tumor that 5'-dFUrd cytotoxicity appears to be dependent on its metabolic conversion to FUra. This is shown in figure 1 which compares the LD50 of 5'-dFUrd measured in each cell line (table 1) to the percent conversion of 5'-dFUrd measured in each cell line (table 2). A definite relationship is apparent between the ability of a cell to metabolize 5'-dFUrd and the cells' sensitivity to 5'-dFUrd. The exception to this was the 47-DN breast cell line. However, as was established above, 5'-dFUrd appears to have a mechanism of activity in these cells that is not present in the other cell lines. Also, 47-DN cells did exhibit a comparatively high rate of 5'-dFUrd metabolism.

Our results demonstrate that 5'-dFUrd has variable cytotoxic activity in human cancer cells of different etiologies. The length of exposure of 5'-dFUrd to these

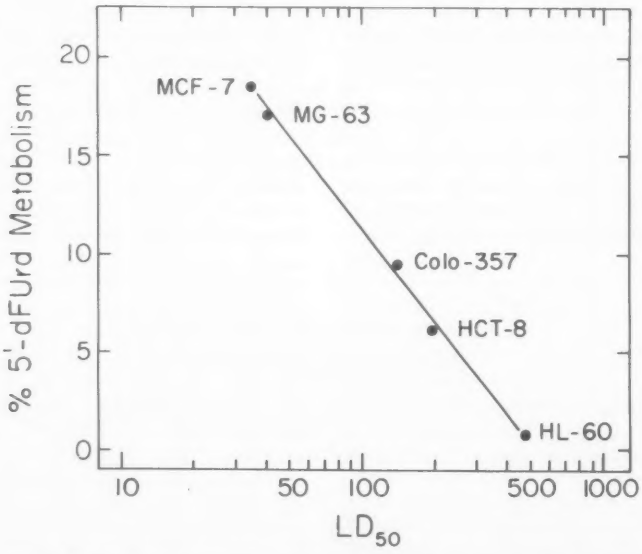

FIGURE 1.—Relationship between 5'-dFUrd metabolism and cytotoxicity. LD50 of 5'-dFUrd measured in each cell line (table 1) is plotted against the % metabolism of 5'-dFUrd in each cell line (table 2).

human lines did not markedly alter the activity of 5′-dFUrd, with the noted exception of the 47-DN cells. It appears that a primary determinant of 5′-dFUrd activity in human cancer cells is the ability of the cell to metabolize 5′-dFUrd to FUra and FUra-nucleotides. The value of 5′-dFUrd as a clinical antineoplastic drug may be limited to a narrow spectrum of tumor types, with cancers of the breast perhaps being responsive to this new fluoropyrimidine.

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Effect of 3-Deazauridine on the Metabolism, Toxicity, and Antitumor Activity of Azacitidine in Mice Bearing L1210 Leukemia Sensitive and Resistant to Cytarabine[1,2]

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The effect of 3-deazauridine (DAUR) on the intracellular purine and pyrimidine nucleotide pools and on the metabolism of azacitidine (aza-CR) in L1210 cells, sensitive (L1210/0) and resistant (L1210/ara-C) to cytarabine (ara-C), was examined. The consequences of such a modulation were correlated with the therapeutic efficacy of this combination in mice bearing L1210 leukemia. In vitro and in vivo treatment of both L1210 sublines with DAUR produced a dose- and time-dependent reduction in the CTP and dCTP pools and an increase in the UTP pool. In addition to these changes in the pyrimidine nucleotide pools, DAUR produced a modest increase in the GTP pool and a marked expansion of the ATP pool in L1210/ara-C 12 hrs following in vivo drug treatment. These perturbations in nucleoside triphosphate pools were more pronounced in L1210/ara-C cells. Treatment of mice bearing L1210/ara-C with 100 mg/kg of DAUR reduced the CTP and dCTP pools in the leukemic cells by > 90% within 1–3 hrs after administration of the drug, with complete recovery of these pools occurring within 12 hrs. Fluctuation of the pyrimidine nucleotide pools after DAUR treatment was correlated with the subsequent increase in aza-CR metabolism and its incorporation into RNA and with the potentiation of the in vivo toxicity of aza-CR. In mice bearing L1210/0 or L1210/ara-C tumors, DAUR or aza-CR produced a ⩽ 23% increase in life-span (ILS). Administration of aza-CR 3 hrs after DAUR, however, produced about an 80% ILS among mice bearing L1210/ara-C tumors, but no more than an approximately 20% ILS among mice bearing L1210/0 tumors. These data suggest that the therapeutic activity of the sequential combination of DAUR and aza-CR against mice bearing L1210/ara-C cannot be explained, per se, on the basis of the initial intracellular modulation of nucleotide pools, since DAUR affected these pools of the two tumors to approximately the same degree. What appears to be important, however, is that such a modulation by DAUR preferentially affected the metabolism of aza-CR in leukemic cells resistant to ara-C which are devoid of deoxycytidine kinase activity. [Cancer Treat Rep 67:547–554, 1983]

The cytidine analog azacitidine (aza-CR) (1,2) inhibits the growth of some experimental and human tumors (3–8). The compound is activated via phosphorylation by uridine-cytidine kinase and is subsequently phosphorylated to the triphosphate derivative, which is then incorporated into RNA. Once incorporation into RNA occurs, aza-CR is capable of inhibiting protein biosynthesis in bacterial and mammalian cells. Although the precise mechanism responsible for this inhibition is not clearly defined, it has been suggested that this cessation of protein synthesis is responsible for the cytotoxic properties of this agent (9–13).

3-Deazauridine (DAUR), a structural analog of uridine (14), inhibits the growth of various strains of bacteria and tumor cell lines in culture and of some experimental tumors in vivo (15–17). Upon entry into cells, DAUR is phosphorylated to the triphosphate which is a potent inhibitor of CTP synthetase (15–17). In experimental sys-

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tems this inhibition is associated with a reduction in the intracellular pools of CTP (15,18).

In L1210 cells resistance to cytarabine (ara-C) is due to an absence of deoxycytidine kinase activity, which results in a decreased intracellular accumulation of ara-CTP (19–21). It has been demonstrated that these ara-C-resistant cells (L1210/ara-C) exhibit collateral sensitivity to DAUR (15,22). The mechanism(s) responsible for the increased sensitivity of these cells to DAUR have not been clearly defined; however, there is some evidence that these cells accumulate higher levels of DAUTP intracellularly than do cells of the ara-C-sensitive parent line (L1210/0), and that CTP synthetase activity in the L1210/ara-C cells is only 50% of that observed in L1210/0 (22).

Recent studies from a number of laboratories suggest that pretreatment of tumor cells with inhibitors of the de novo pyrimidine nucleotide pathway can enhance the metabolism of subsequently administered nucleoside analogs, and can result in synergistic cell killing (23). For example, it has been shown that DAUR pretreatment enhances the metabolism and the cytotoxicity of aza-CR in L5178Y cells (24). A schematic representation of the metabolic pathway of aza-CR and sites at which metabolic modulation by DAUR may occur is shown in figure 1. According to this scheme, DAUR pretreatment could potentially produce a cascade of events leading to a decrease in the intracellular pools of CTP and dCTP, resulting in an enhanced metabolic activation and incorporation of aza-CR into RNA. A depletion of the CTP pool might conceivably enhance the metabolism of aza-CR, since CTP is a known physiologic feedback inhibitor of uridine-cytidine kinase which catalyzes the rate-limiting step in the activation of aza-CR (24–26).

The present studies were undertaken to determine whether the enhancements of aza-CR metabolism produced by DAUR in target cells with a specific metabolic defect in pyrimidine metabolism (ie, L1210/ara-C) can, in fact, result in the enhancement of the therapeutic efficacy of aza-CR.

MATERIALS AND METHODS

Chemicals and Radioactive Compounds

[4-^{14}C]aza-CR was purchased from Moravek Biochemical Corp (specific activity, 50 mCi/mmol; radiochemical purity, 97%). Aza-CR and DAUR were obtained from the National Cancer Institute, Bethesda, MD.

High-Performance Liquid Chromatography (HPLC)

The HPLC system described previously for the separation of intracellular ara-C metabolites (27) was used for the separation of intracellular ribonucleotides and aza-CR metabolites derived from ^{14}C-aza-CR. The retention times were 6.0, 18.0, and 28.5 mins for the mono-, di-, and tri-phosphate derivatives of aza-CR, respectively. Following the injection of 10–20 μl of radio-labeled acid-soluble extract onto the HPLC column, 1.5-min fractions were collected into vials. 3a70 liquid scintillation fluid (10 ml) was added to the vials, and the radioactivity was counted in a Packard Tricarb Model B2450 liquid scintillation spectrometer, with a counting efficiency of 85% for ^{14}C.

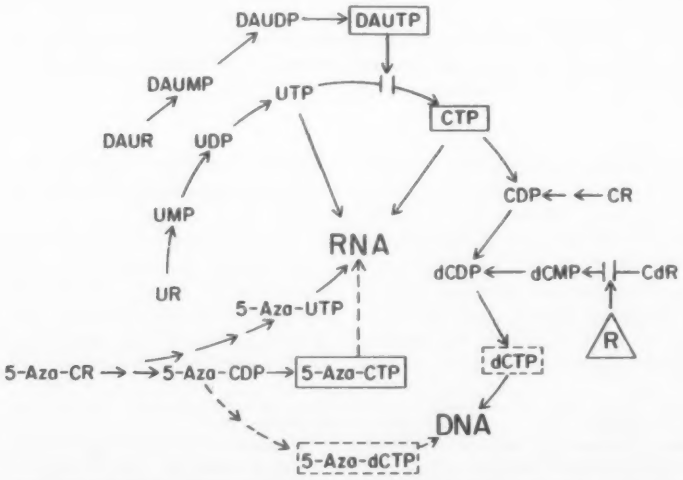

FIGURE 1.—Metabolic pathways for pyrimidine nucleosides, DAUR, and aza-CR. Basis of resistance to ara-C in L1210/ara-C and site of action of DAUTP are also shown.

In Vitro Effect of DAUR Pretreatment on the Metabolism of Aza-CR

L1210/ara-C or L1210/0 cells were removed from mice 3 days after ip implantation of 1.0×10^6 cells. Rbcs were removed by lysis, and the remaining cells were centrifuged and suspended in RPMI-1640 medium containing 10% fetal calf serum (5×10^6 cells/ml) and incubated at 37°C with 2.0 or 10 μM DAUR for 3 hrs. The cells were then washed three times with cold 0.9% NaCl solution and were resuspended in fresh medium containing [^{14}C]aza-CR (7.25 μM, 1 μCi/ml). At 20, 40, and 60 mins, thereafter, 2-ml aliquots were removed, centrifuged at $100 \times g$ for 1 min, and the cell pellets were washed three times with cold 0.9% NaCl. The cells were extracted with 0.3 ml of 95% methanol for 2 mins at 4°C, and 0.2 ml of distilled water was added. After centrifugation at $1000 \times g$ for 5 mins and evaporation of the supernatant under a stream of nitrogen, the residue was reconstituted in 100 μl of H_2O and 10–20-μl aliquots of the solution were injected onto an HPLC column for analysis. The methanol-precipitable fractions were washed once with methanol and three times with 6% perchloric acid (PCA). The amount of drug incorporated into RNA and DNA was quantitated by hydrolysis of the macromolecular fraction with 0.5 N NaOH at 4°C overnight. The hydrolysate was then neutralized to pH 7 and centrifuged at $1000 \times g$ for 5 mins. An aliquot of the supernatant (containing RNA) was then counted for radioactivity, while the DNA-containing pellet was washed three times with 6% PCA, and resuspended in PCA and heated at 95°C for 15 mins. Following centrifugation an aliquot of the supernatant (containing hydrolyzed DNA) was removed and counted for radioactivity. RNA and DNA determinations were performed as described previously (28).

Effect of DAUR Pretreatment on the CTP and dCTP Pools of L1210/0 and L1210/ara-C Cells

In vivo effects.—DBA/2J mice bearing L1210/ara-C or L1210/0 tumor cells (3 days after ip transplantation of 10^6 cells) were infused in the tail vein (29,30) for 24 hrs with DAUR at doses of 30, 60, or 90 mg/kg/day. The control groups were infused with 0.9% NaCl for 24 hrs. Following the infusion, three mice per group were killed by cervical dislocation. Cells were harvested from the peritoneal cavity, washed twice with 0.9% NaCl solution, and counted in a 0.1% trypan blue solution (in saline) to assess viability. Cells were then extracted with 95% methanol, centrifuged at $1000 \times g$ for 5 mins, and the methanol-soluble fraction was evaporated under a stream of nitrogen. The residue was reconstituted in H_2O and analyzed by HPLC for ribonucleotide pools as described above, while the levels of the deoxyribonucleoside triphosphate pools were determined by the DNA polymerase assay (31).

In vitro effects.—Tumor cells (3 days after ip transplantation of 10^6 cells in DBA/2J mice) were harvested, washed free of rbcs, counted, and incubated with DAUR (2 and 10 μM) for 3 hrs. Cells were then centrifuged, washed three times with cold 0.9% NaCl, and centrifuged at $100 \times g$ for 2 mins; the cell pellet was then extracted with 95% methanol. Following centrifugation the methanol-soluble supernatant was evaporated under a stream of nitrogen. The residues were reconstituted in H_2O and the ribo- and deoxyribo-nucleoside triphosphate pools were quantitated as described above.

Toxicity and Antitumor Activity

The toxicity and antitumor activity of DAUR and aza-CR were determined in female DBA/2J mice. In the toxicity studies DAUR was administered as a continuous iv infusion for 24 hrs, and aza-CR was administered as a 3-hr infusion. Toxicity was evaluated on the basis of weight loss and mortality. In the chemotherapy studies drug treatment was initiated 24 hrs after the mice were injected ip with 1.0×10^6 cells. In these experiments both drugs were administered as single ip bolus injections.

RESULTS

Effects of DAUR on Nucleoside Triphosphate Pools

Figure 2 demonstrates that DAUR produced a dose-dependent decrease in the CTP pools of L1210/0 and L1210/ara-C in the in vivo and the in vitro experiments; however, this effect was consistently more pronounced in the ara-C-resistant cells. The in vitro data indicated that the maximal effect of DAUR on the cytidine nucleotide pools was achieved following a 3-hr incubation with the drug (data not shown).

The data in figure 3 demonstrate the in vivo effects of DAUR on the intracellular nucleotide pools of L1210/ara-C. A maximum reduction in the CTP and dCTP pools was achieved within 3 hrs, although both pools recovered completely within 12 hrs following drug treatment. Twelve hrs after DAUR treatment there was still a significant expansion of the ATP and UTP pools in the leukemic cells, while the GTP pool was increased only slightly above control values obtained from saline-treated mice.

Aza-CR Metabolism by Leukemic L1210 Cells

Table 1 and figure 4 compare aza-CR uptake and metabolism in L1210/0 and L1210/ara-C. Table 1 shows that the intracellular pool of aza-CTP and its incorporation into RNA were approximately twofold greater in L1210/ara-C than in L1210/0 following a 1-hr exposure to aza-CR; however, the extent of drug incorporation into DNA was similar in both cell lines.

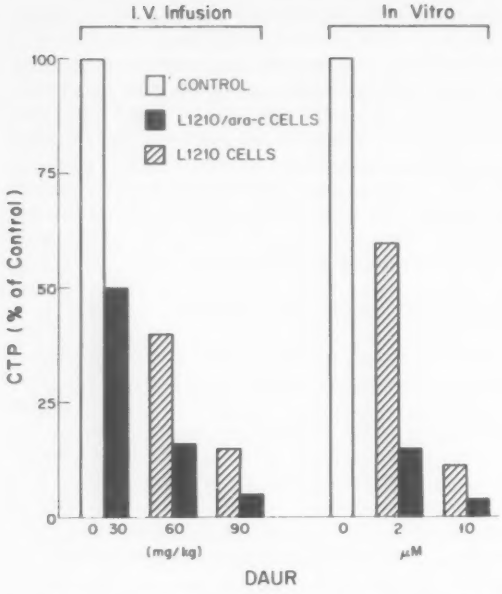

FIGURE 2.—Effects of DAUR on the CTP pools of L1210/ara-C and L1210/0 cells. For the iv infusion, L1210 cells were removed from the peritoneal cavity of mice immediately after the termination of a 24-hr continuous iv infusion of DAUR. For the in vitro experiments, L1210 cells were incubated with DAUR for 3 hrs at the designated concentrations. For both conditions, controls were designated as 100%. CTP pools for untreated L1210/0 and L1210/ara-C were 2.0 ± 0.6 and 1.9 ± 0.7 nmols/10^7 cells, respectively.

Effects of DAUR on the Metabolism of Aza-CR

The data in figures 2 and 4 demonstrate that in L1210/ara-C a 3-hr pretreatment with 2.0 μM DAUR produced a marked depletion of the CTP pool, and significantly enhanced aza-CR uptake by these cells. Aza-CR uptake by these cells was not increased above control values when the concentration of DAUR was increased to 10.0 μM, despite the fact that the CTP pool was decreased to < 10% of control. In the parent line, L1210/0, DAUR produced a dose-dependent decrease in the CTP pool; however, neither concentration of DAUR was able to enhance aza-CR uptake by this tumor.

Incorporation of Aza-CR into RNA and DNA

The effects of DAUR pretreatment on the subsequent incorporation of aza-CR into the RNA and DNA of L1210/0 and L1210/ara-C cells are shown in figure 5. DAUR pretreatment doubled the incorporation of aza-CR into the RNA of L1210/0; however, this effect was even more pronounced in L1210/ara-C, especially at 2.0 μM DAUR. DAUR pretreatment had no significant effect on the incorporation of aza-CR into the DNA of either cell line.

Toxicity Studies

Figure 6 illustrates the effect of DAUR on aza-CR toxicity in normal mice. When aza-CR was administered alone by continuous infusion for 3 hrs, the LD50 was approximately 210 mg/kg/3 hrs; however, when the animals were pretreated with a nontoxic dose of DAUR, 60 mg/kg/day for 24 hrs (31), the LD50 of aza-CR was reduced to approximately 60 mg/kg/3 hrs. Thus, DAUR pretreatment potentiated the toxicity of aza-C threefold to fourfold in normal animals.

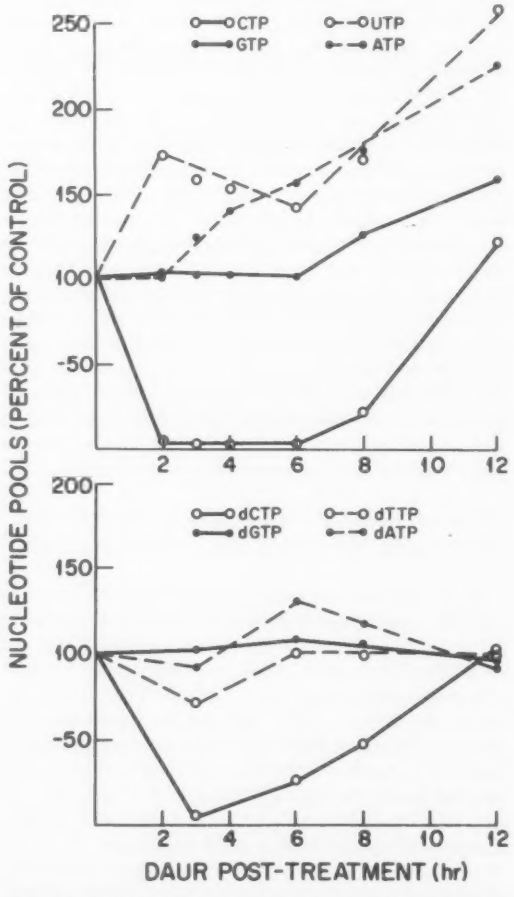

FIGURE 3.—Effect of DAUR on the ribonucleoside and deoxyribonucleoside triphosphate pools of L1210/ara-C in vivo. Cells were removed from mice bearing leukemia L1210/ara-C at various times following a single ip injection of DAUR (100 mg/kg). Data are expressed as % control values of nucleotide pools in saline-treated mice.

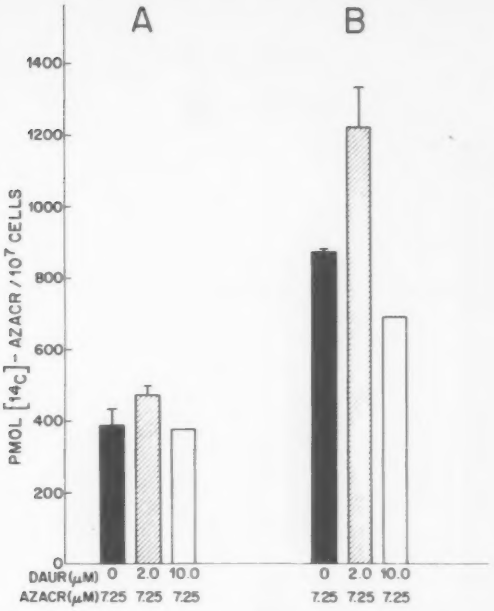

FIGURE 4.—In vitro effects of DAUR pretreatment on the intracellular accumulation of ^{14}C-aza-CR and its metabolites. Cells were incubated with and without DAUR (2.0 or 10 μM at 37°C for 3 hrs followed by a 1-hr incubation with ^{14}C-aza-CR, 7.25 μM). A = L1210/0; B = L1210/ara-C.

This figure also shows that the dose response curve for aza-CR toxicity became steeper when the animals were pretreated with DAUR. An even greater shift in the dose response curve was obtained when both DAUR and aza-CR were infused together for 3 hrs following pretreatment with a 24-hr infusion of DAUR alone (data not shown).

Antitumor Activity of the Sequential Combination of DAUR and Aza-CR

Table 2 and figure 7 summarize the antitumor activity of DAUR and aza-CR (when administered alone or in combination) in female DBA/2J mice bearing leukemia L1210/0 or L1210/ara-C. Table 2 shows that no treatment regimen in which the drugs were administered alone or concurrently possessed any significant antitumor activity against L1210/ara-C; however, when aza-CR was administered 3 hrs after DAUR treatment, considerable antitumor activity was noted against the tumor, since there was an 80% increase in lifespan (ILS) in tumor-bearing animals. In contrast, neither this regimen nor treatment with DAUR or aza-CR alone produced a > 20% ILS in mice bearing L1210/0.

DISCUSSION

Since DAUR is capable of modulating the intracellular nucleotide pools, it was of interest to evaluate the effect of this modulation on the metabolism of aza-CR, and on the therapeutic outcome of a DAUR/aza-CR sequential combination in DBA/2J mice bearing leukemia L1210/0 and L1210/ara-C. In the in vitro experiments shown in figure 2, DAUR treatment produced a dose-dependent reduction in the CTP pools of L1210/0 and L1210/ara-C, although the extent of this reduction was greater in the ara-C-resistant tumor. DAUR treatment of L1210/ara-C-bearing mice (fig 3) produced rapid reduction in the CTP and dCTP pools of the leukemic cells, an expansion of the UTP pool, and a more delayed increase in the purine ribonucleoside triphosphate pools. The data presented in figure 3 further suggest that the effect of DAUR on the CTP pool precedes its effect on the dCTP pool, although both nucleotide pools recovered completely within 12 hrs following DAUR treatment.

It has been reported that a depletion of the intracellular CTP pool, the natural feedback inhibitor of uridine-cytidine kinase, might result in an enhanced phosphorylation of aza-CR (24–26). Figures 4 and 5 demonstrate that the effect of DAUR on the intracellular uptake and metabolism of aza-CR was significantly more pronounced in L1210/ara-C than in L1210/0. Perhaps only in the L1210/ara-C cells was the reduction in the cytidine nucleotides sufficient to enhance aza-CR uptake. Alternatively, these data may suggest that a depletion of the CTP pool, per se, is not the most critical factor in the ability of DAUR to enhance the metabolism of aza-CR.

TABLE 1.—In vitro metabolism of ^{14}C-aza-CR by L1210/0 and L1210/ara-C cells*

Cell lines	Intracellular aza-CTP pool (pmols/10^7cells)	Aza-CTP incorporation (pmols/μg) into— RNA	DNA
L1210/ara-C	325.1 ± 69	2.9 ± 1.8	1.4 ± 0.5
L1210/0	179.8 ± 21	1.5 ± 0.6	1.4 ± 0.8
Ratio of L1210/ara-C to L1210/0	1.8	1.9	1.0

* Tumor cells were incubated for 1 hr with the drug and the acid-soluble and -insoluble fractions were prepared as described in the Materials and Methods section.

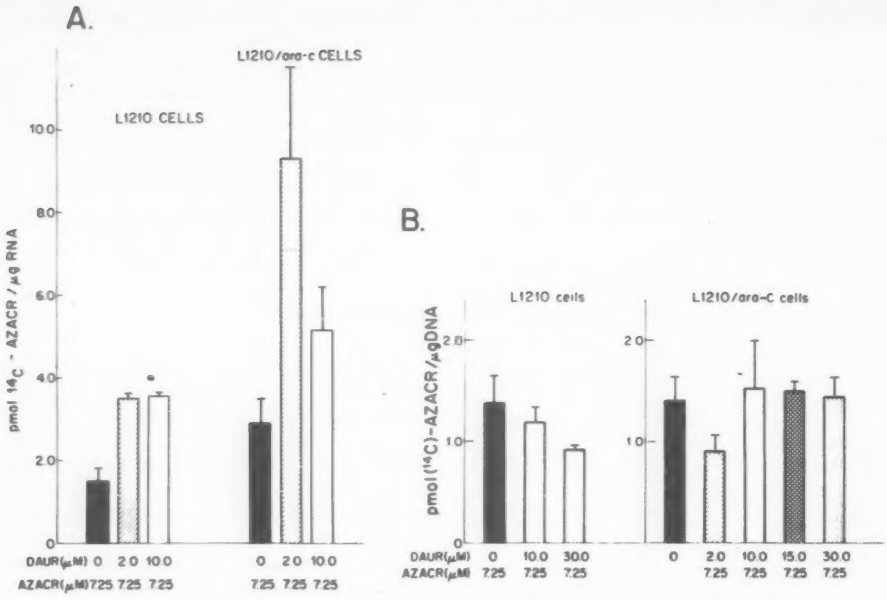

FIGURE 5.—Effects of DAUR pretreatment on the incorporation of ^{14}C-aza-CR into RNA (A) and DNA (B) of L1210/0 and L1210/ara-C cells. Cells were incubated with and without DAUR (2.0 or 10 µM) at 37°C for 3 hrs followed by 7.25 µM ^{14}C-aza-CR (1 µCi/ml) for 1 hr.

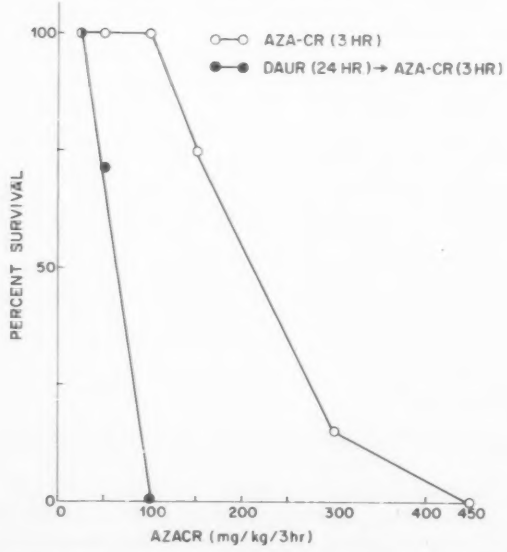

FIGURE 6.—Toxicity of aza-CR alone and in combination with DAUR. Normal mice were treated with DAUR (60 mg/kg) or saline by a 24-hr continuous infusion followed by a 3-hr infusion of aza-CR at various doses.

What may be more important is the differential sensitivity of the two cell lines to the metabolic perturbations in the endogenous levels of the nucleoside triphosphate pools which are produced by DAUR treatment. Thus, although DAUR could reduce the CTP and dCTP pools in both tumors, this reduction may have been more critical for the L1210/ara-C cells. The L1210/0 cells might possess efficient capability of utilizing exogenous cytidine and deoxycytidine to restore the cytidine nucleotide pools to control values following DAUR treatment. In contrast to this situation, the L1210/ara-C tumor would be unable to salvage deoxycytidine. Thus, these ara-C-resistant cells might rely more extensively on an enhanced salvaging of cytidine to restore their CTP and dCTP pools (through the enzyme ribonucleotide reductase). A DAUR pretreatment of this tumor might also enhance aza-CR uptake and metabolism by these cells since this cytidine analog is activated via the same pathways as cytidine. An agent such as DAUR, which can perturb the intracellular levels of the ribo- and deoxyribo-nucleotide pools, might have different effects on the two tumors regarding the regulation of pyrimidine nucleotide metabolism. Thus, in the present studies, the inability of L1210/ara-C to salvage deoxycytidine may be partially responsible for the finding that DAUR enhanced aza-CR uptake, metabolism, and antitumor activity in the ara-C-resistant line to a greater extent than it did in the parent line. A some-

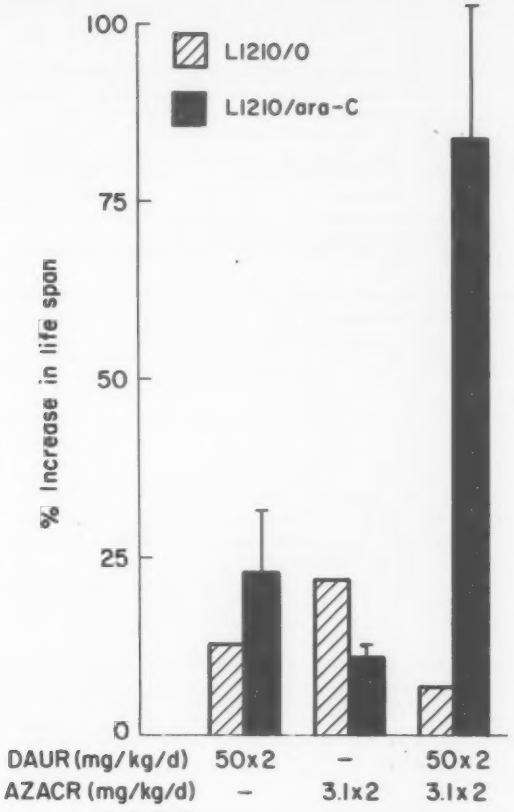

FIGURE 7.—Antitumor activity of the sequential combination of DAUR and aza-CR. DBA/2J mice were injected ip with 1.0×10^6 L1210 cells and treatment was initiated 24 hrs later. DAUR (50 mg/kg) and aza-CR were administered ip daily for 2 days. On both days of therapy aza-CR was administered 3 hrs after administration of DAUR.

what curious finding is that 2.0 μM DAUR enhanced aza-CR uptake in L1210/ara-C to a greater extent than did 10.0 μM DAUR (figs 4 and 5). The results are in general agreement with those reported by Grant and Cadman (24), who suggested there may be competition betwen DAUR and aza-CR for intracellular metabolism.

Table 2 demonstrates that no treatment consisting of DAUR or aza-CR administered alone or concurrently possessed any significant antitumor activity against L1210/ara-C; however, a marked ILS among tumor-bearing animals was noted when aza-CR was not given until 3 hrs after DAUR treatment. This 3-hr delay may be sufficient for the host normal tissues, but not the tumor cells, to recover from the biochemical effects induced by DAUR. Thus, this treatment schedule would impart some selectivity to this drug combination. In L1210/0, waiting 3 hrs between the DAUR and aza-CR treatments is not a

TABLE 2.—Antitumor activity of DAUR and aza-CR when given alone or sequentially against L1210/ara-C leukemia in DBA/2J mice

Treatment	Dose (mg/kg/day)	Survival in days (mean ± SD)
Saline	—	7.5 ± 0.5
DAUR	100 × 2	9.7 ± 1.3
DAUR	60 × 1	9.3 ± 1.2
DAUR	50 × 1	9.3 ± 1.7
DAUR	50 × 2	10.2 ± 1.3
Aza-CR	25 × 1	9.0 ± 2.4
Aza-CR	12.5 × 2	8.4 ± 1.8
Aza-CR	3.1 × 2	8.4 ± 1.2
DAUR	50 × 2	8.5 ± 0.8
Aza-CR*	3.1 × 2	
DAUR	50 × 2	11.4 ± 0.5
Aza-CR†	3.1 × 2	
DAUR	50 × 2	13.8 ± 1.2
Aza-CR‡	3.1 × 2	

*Concurrent administration.
†1-hr interval.
‡3-hr interval.

sufficient time to allow the CTP pool of the leukemic cells to recover from the effects of DAUR (32); however, this regimen possessed no antitumor activity against the parent line. The results of these chemotherapy studies are consistent with the notion that it is the inability of L1210/ara-C cells to salvage deoxycytidine, and not the DAUR-induced depletion of the cytidine nucleotides, per se, which may be responsible for this synergistic antitumor activity of this sequential DAUR/aza-CR combination against this tumor.

In summary, the data presented herein indicate that the sequential administration of DAUR followed by aza-CR is of greater therapeutic value against leukemia L1210 resistant to ara-C than against the parent line. Scheduling of the two agents based on the biochemical and pharmacologic data obtained may assist in the therapeutic utilization of this combination in cancer chemotherapy.

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Activity and Pharmacokinetics of Teniposide in Lewis Lung Carcinoma-Bearing Mice[1,2]

Massimo Broggini, Tina Colombo, and Maurizio D'Incalci[3,*]

The antitumor activity of teniposide (VM26) was investigated in Lewis lung carcinoma (3LL) of the mouse after a single dose of 20 mg/kg iv (given 8 or 14 days after tumor transplant) or after three doses of 6.5 mg/kg iv (given on Days 8, 11, and 14 after tumor transplant) (total dose, 19.5 mg/kg). The single dose resulted in only 25% primary tumor reduction but had marked antimetastatic activity. The repeated doses (6.5 mg/kg × 3) were much more effective, with 85% primary tumor reduction and the apparent disappearance of all metastatic deposits. The pharmacokinetics of VM26 was investigated in 3LL-bearing mice by a high-performance liquid chromatographic assay. At both of the above doses VM26 disappeared from mouse plasma biphasically, with an elimination half-life of about 70 mins. The concentrations in metastases were higher than in primary tumor, where very low levels were found. The highest VM26 levels were found in liver, small intestine, and kidney, and the lowest levels were found in the brain. Excretion of VM26 in urine amounted to < 5% of the dose. [Cancer Treat Rep 67:555–559, 1983]

The semisynthetic derivative of epipodophyllotoxin, teniposide (VM26), has gained importance because of its effectiveness against a variety of experimental and clinical tumors (1,2). To our knowledge, the only data available regarding the disposition of VM26 in laboratory animals were obtained in the rat using relatively nonspecific methods such as radioisotopic assay after treatment with ^{3}H-VM26 (3). No information is yet available on the disposition of VM26 in the mouse, the species most used for preclinical studies aimed at clarifying the mode of action and the activity of anticancer agents. Since a knowledge of VM26 pharmacokinetics could prove useful in planning better investigations and making better therapeutic use of this drug, we studied the antitumor activity of VM26 in various treatment schedules and its pharmacokinetics in Lewis lung carcinoma (3LL)-bearing mice.

MATERIALS AND METHODS

Animals and tumor.—Male C57BL/6 mice (body weight, 20 ± 2 g), from Charles River Breeding Laboratories, Italy, were used for these studies. The experimental tumor employed was the 3LL implanted im (10^{5} viable cells/0.1 ml/mouse), which induces macroscopic metastases in the lungs. The 3LL was maintained in the same strain of mice by im transplantation every 2 weeks.

Drug and experimental design.—VM26, supplied by Bristol-Myers, NY, was dissolved in DMSO:Tween 80:saline (0.5:1:8.5). For assessment of antitumor activity, mice were given a single iv dose of 20 mg/kg of VM26 (8 or 14 days after tumor implantation) or three doses of 6.5 mg/kg iv (on Days 8, 11, and 14 after tumor implantation). Control animals received an iv injection of the solution used to dissolve the drug. Ten animals per group were killed 25 days after the 3LL inoculation and tumor and metastasis weights were recorded. A second group of animals were used to record survival time. Statistical significance of the differences between values for controls and treated groups was assessed by Duncan's test. For pharmacokinetic studies, mice bearing 25-day 3LL were given VM26 in a single iv dose of 6.5 or 20 mg/kg. Four animals per time were killed 1, 5, 15, 30, 60, 120, 180, and 360 mins after injection, and plasma and tissues (tumor, metastases, liver, kidney, spleen, small intestine, heart, and brain) were removed and frozen at −20°C until analyzed. For determination of drug excretion in urine, mice were housed in metabolic cages and urine was collected for

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15, 24, and 48 hours after drug administration.

Analytic assay.—Plasma, urinary, and tissue levels of VM26 were determined as described (4). Fifty microliters of etoposide (VP16) (supplied by Bristol-Myers, NY) was added, as internal standard, to 0.2 ml of plasma or urine or 1 ml of tissue homogenate (1:5 in water; obtained using an Ultra Turrax apparatus, Model TP18-10, Jane and Kungel, Staufen, W Germany). VP16 was used as internal standard since it is structurally similar to VM26; it gives good high-performance liquid chromatographic separation from VM26 and other possible metabolites (ie, picro-VM26 and hydroxyacid-VM26) and is not a metabolite of VM26. After extraction with 8 ml of chloroform, the samples were dried under vacuum and then redissolved with 100 μl of mobile phase. This solution (5–20 μl) was injected into a Waters Model 6000A high-performance liquid chromatograph equipped with a 254-nm absorbance detector. An isocratic solvent system of water (60%), acetonitrile (39%), and acetic acid (1%) was used for separation at a flow rate of 1 ml/min using a micro-Bondapak phenyl column 25 cm in length (Merck). Recovery was 80% ± 2% and sensitivity was 0.2 μg/ml of plasma or 0.5 μg/g of tissue.

Pharmacokinetic analysis.—Pharmacokinetic parameters in plasma were calculated by the peeling method assuming a two-compartment open model using a Hewlett-Packard Model 9810 H calculator.

RESULTS

Table 1 shows the chemotherapeutic activity of VM26 in three different dose schedules. The dose schedule of 6.5 mg/kg iv for 3 days (total dose, 19.5 mg/kg) resulted in a marked reduction of tumor weight (about 80%). Little reduction (25%) was observed after a single dose of 20 mg/kg at two different times after tumor implantation.

The antimetastatic effects were striking in all the dose schedules. Better antimetastatic activity was seen with the divided dose, all of the animals being apparently free of lung metastatic involvement. The superiority of the split-dose schedule is confirmed by longer survival time. Figure 1 shows the plasma disappearance curve of VM26 in the 3LL-bearing mice after iv injections of 6.5 and 20 mg/kg. At both doses the rate of disappearance appeared to be biphasic with a very rapid initial decline followed by a second, much slower phase starting approximately 15 minutes after injection. The half-lives for both treatments were similar (the α-half-life was about 2 mins and the β-half-life was about 70 mins). The zero time concentrations were proportional to the doses; the area under the curve values were slightly less than proportional, being 711 and 1608 μg/ml × min after doses of 6.5 and 20 mg/kg, respectively.

Figures 2 and 3 show that distribution of VM26 in different tissues was similar at the two dose levels. High drug concentrations were found in the liver, kidney, and small intestine. Levels were lowest in the brain. The peak concentrations in metastases were about four times those in the primary tumor, where drug levels were low.

Excretion of VM26 in the urine as unchanged drug in the 48 hours after iv injection of 6.5 or 20 mg/kg corresponded to 3.4% and 4.6%, respectively, of the dose administered; > 90% was eliminated in the first 24 hours.

DISCUSSION

As already demonstrated in other mouse tumors (1), 3LL treated with VM26 also shows dose schedule-dependent antitumor activity. Administering the total dose of 20 mg/kg in several doses gives a much better antitumor effect and longer survival time than giving the same amount in a single dose. This schedule de-

TABLE 1.—Antitumor and antimetastatic activity of VM26 in im 3LL tumor-bearing mice*

Treatment (mg/kg iv)	Toxic deaths (%)	Mean primary tumor weight (g) ± SE	Metastasis-free mice (%)	Lung metastases		Median survival (days)
				Mean No./ mouse ± SE	Mean weight (mg)/ mouse ± SE	
—	0	8.658 ± 0.499	0	21 ± 4	120 ± 42	25.0
20 × 1 (Day 8)	0	6.440 ± 0.225	78	2 ± 1	5 ± 4	33.0
20 × 1 (Day 14)	20	6.502 ± 0.357	13	3 ± 1	12 ± 7	32.5
6.5 × 3 (Days 8, 11, and 14)	0	1.635 ± 0.206†	100	0	—	40.0

* Each group consisted of 10 mice; antitumor and antimetastatic activity was evaluated 25 days after im transplantation of 10^6 tumor cells in C57BL/6 mice.

†$P < 0.01$ (Duncan's test).

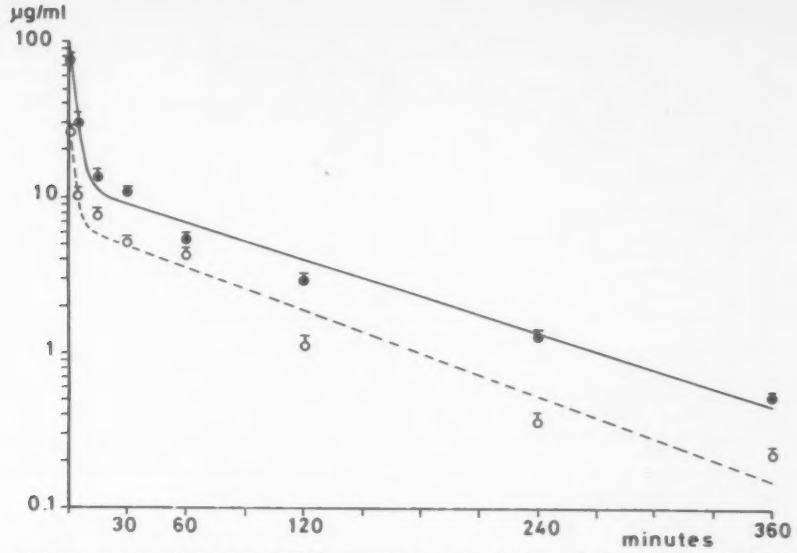

FIGURE 1.—Semilogarithmic plots of VM26 concentration vs time in plasma of mice bearing im 3LL (25 days) after iv injection of 6.5 mg/kg (o----o) or 20 mg/kg (•——•).

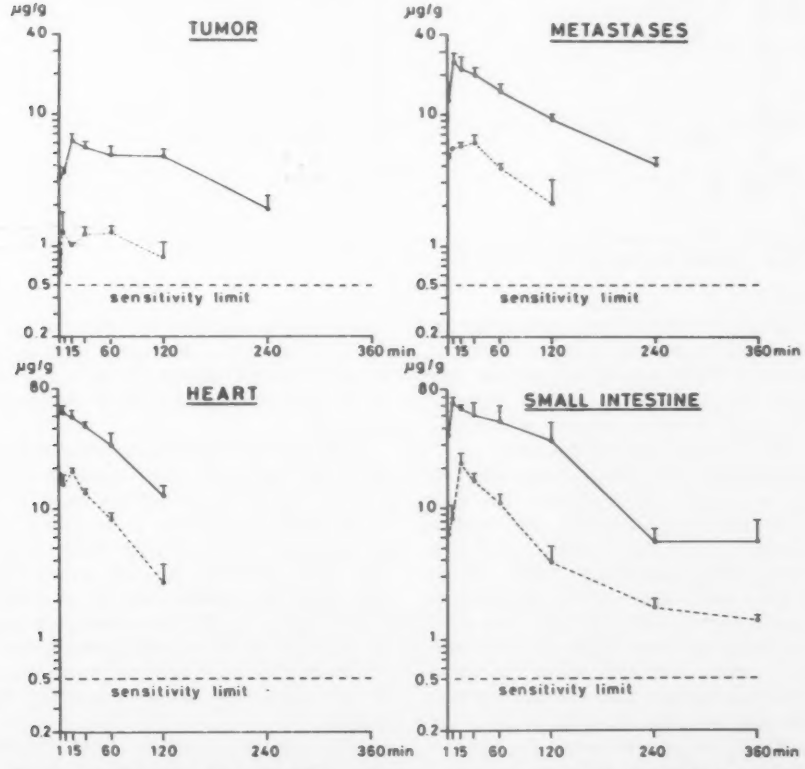

FIGURE 2.—Semilogarithmic plots of VM26 concentrations vs time in tissues of mice bearing im 3LL (25 days) after iv injection of 6.5 mg/kg (•----•) or 20 mg/kg (•——•).

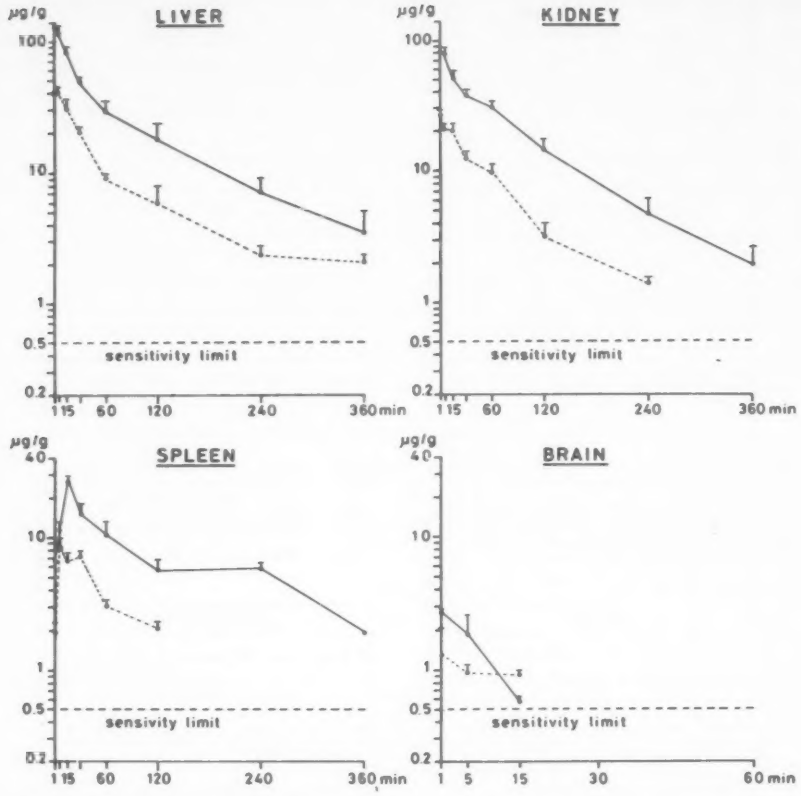

FIGURE 3.—Semilogarithmic plots of VM26 concentrations vs time in tissues of mice bearing im 3LL (25 days) after iv injection of 6.5 mg/kg (•-----•) or 20 mg/kg (•——•).

pendence, already seen for other drugs, is probably related to the phase-specific cytotoxic effects of VM26. It has been shown that VM26 acts in S-G_2 phases, preventing the cells from entering mitosis (5); repeated doses, therefore, have a better chance of reaching larger cell populations in the susceptible phases.

As already reported (6), VM26 has a greater effect on metastases than on primary 3LL carcinoma. This may be related to the fact that metastatic cells have a larger growth fraction with a higher percentage of cells in cycle and thus possibly susceptible to VM26. An additional reason is the much higher VM26 concentration found in lung metastatic deposits. At both doses peak levels were more than four times higher in metastases than in primary tumor, and area under the curve values were more than doubled. This different distribution has already been described for other drugs in the same animal model (7) and may be due to the reported better blood supply of metastases. However, for a lipophilic compound such as VM26, the extraction of tissues should be negligibly influenced by the tissue blood supply. Therefore, other reasons such as a larger number of viable cells per unit of weight or more efficient uptake mechanisms might be at the basis of this phenomenon.

VM26 concentrations in the tumor are low, but probably high enough to exert cytotoxic effects, as suggested by the antitumor activity data. The low levels of VM26 in the mouse brain agree with the finding that in human cerebrospinal fluid VM26 is not detectable after drug treatment (8). This finding conflicts, however, with the reported activity of VM26 in human CNS malignancies (2). The discrepancy may be due to the presence of as yet unidentified active metabolites which cross the blood-brain barrier or to a modification of the blood-brain barrier caused by human brain tumors.

VM26 appears to be more highly concentrated in the liver, small intestine, and kidney, organs possibly involved in elimination of the drug. The high concentra-

tions in the kidney, however, do not imply large-scale renal excretion of VM26. In fact, elimination of VM26 in urine amounted to < 5% of the dose in the 48 hrs after drug injection, a sufficiently long period considering that the plasma elimination half-life is approximately 70 mins. It is worth noting that scant urinary elimination of VM26 as unchanged drug has been recently reported in humans also (8). How VM26 is cleared from the body remains unclear. A possibility to be investigated is biliary excretion, and the metabolism of VM26 might also be implicated in VM26 clearance. In our high-performance liquid chromatograph analysis after extraction with chloroform, no peaks corresponding to possible metabolites of VM26 were observed, but there may be metabolites that are not extractable with chloroform, such as the hydroxy acid VM26 (4) or conjugated bioproducts recently identified for the analog VP16 in urine of patients (N. D'Incalci et al, unpublished data).

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Preclinical Re-evaluation of Benzaldehyde as a Chemotherapeutic Agent[1,2]

Raymond Taetle* and Stephen B. Howell[3]

Benzaldehyde (BA), an agent extracted from figs, is reported to have antitumor activity in vitro against a variety of experimental tumors and in vivo against refractory human neoplasms. We employed recently developed in vitro techniques to examine the effect of BA on growth of malignant human cells, and examined its effects in vivo against two human tumor xenografts established from primary specimens. BA showed dose-dependent inhibition of HL60 promyelocytic leukemia cells and normal human granulocyte/macrophage colonies. It showed no in vitro activity against either KG-1 myeloid leukemia cells or chronic lymphocytic leukemia cells grown in colony culture. BA failed to inhibit growth of either HL60 or KG-1 cells in liquid culture, and did not induce differentiation of HL60 cells. When tested against human tumor colony-forming cells from 30 patients with solid tumors, inhibition of colony growth > 70% was seen in six patients (20%). BA failed to inhibit the in vivo growth of either the T222 epidermoid carcinoma xenograft or the T380 ovarian carcinoma xenograft. We conclude that BA lacks significant activity against most human tumors tested in these experimental systems. [Cancer Treat Rep 67:561–566, 1983]

Each year, the National Cancer Institute (NCI) (Bethesda, MD) screens thousands of compounds for antitumor activity, using a panel of in vivo and in vitro animal tumor models (1). Recently, nude mouse xenografts and in vitro human tumor colony-forming assays have been added to these screening procedures on a trial basis (1–3). Previous evaluations of the NCI screening process showed a high false-positive rate, but indicated probable detection of the vast majority of active compounds (1). However, the false-negative rate of the system (exclusion of active drugs) remains unknown, since agents showing no activity are not tested in clinical trials.

Extracts of figs have been reported to show antitumor activity, with the active principal being benzaldehyde (BA), a periactin which may be isolated from figs by steam distillation (4). BA was previously evaluated by the NCI in several forms, and was not found to have significant antitumor activity (M. K. Wolpert, personal communication). However, Takeuchi et al (4) and Zundel et al (5) have reported antitumor activity of BA in several experimental systems, and Kochi et al (6) have reported a high degree of clinical activity for this compound in heavily pretreated patients with cancer. Because of these reports, we undertook a re-evaluation of BA using recently developed experimental in vitro and in vivo screening systems for human tumors. After testing the compound against a wide variety of human neoplasms, we find that BA lacks activity against most human tumors.

METHODS

Benzaldehyde

BA was supplied as benzaldehyde-glucose by Dr. M. Kochi (Ichijo-Kai Hospital, Chiba, Japan) and was solubilized as instructed in normal saline, pH 8.0. Stock solutions were diluted with saline just prior to plating and were added directly to in vitro cultures. BA doses and concentrations were calculated exclusive of the glucose moiety. For in vivo studies, animals were inoculated ip daily at doses of 25 or 50 mg/kg. The toxic (LD10) dose for BA was > 200 mg/kg.

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Hematologic Neoplasms

The effects of BA were assessed on normal bone marrow granulocyte/macrophage progenitors, HL60 promyelocytic leukemia cells, KG-1 myeloid leukemia cells, and chronic lymphocytic leukemia (CLL) colony-forming cells (CFC). Granulocyte/macrophage progenitors (CFU-GM) were grown as previously described (7). The final culture mixture contained 2×10^5/ml bone marrow mononuclear cells, Iscove's modification of Dulbecco's MEM, 15% fetal calf serum (FCS), and 20% placenta-conditioned medium in 0.3% agar. Cultures were incubated for 10 days in 7.5% CO_2, and total clusters (aggregates of 10-40 cells) and colonies (> 40 cells) were scored using an inverted microscope.

KG-1 and HL60 leukemia cell lines (8) were grown in colony culture as previously described (7). KG-1 cells were supplied by Dr. Carlos Izaguirre (Ontario Cancer Institute, Toronto, Canada). The final culture mixture contained 20,000 cells/ml in Iscove's MEM and 15% FCS in 0.3% agar. The cultures were incubated as described above, and aggregates of > 40 cells were scored as colonies after 10-14 days of incubation. Liquid cultures of HL60 and KG-1 cells were also used to study the effects of BA. Cells were maintained in RPMI-1640 and 10% FCS, and cultures were initiated with $1\text{-}5 \times 10^5$ cells/ml with BA at 50 or 100 μg/ml when in log (growth) phase. Media were not changed during the study. Viable cell counts were performed daily, using trypan blue dye exclusion. HL60 cells were assessed for possible effects of BA on differentiation by sampling cultures and preparing cytospins for morphology.

CLL CFC were grown as previously described (9). The final culture mixture contained 4×10^5/ml T-cell-depleted CCL cells in Iscove's medium, 3×10^5/ml irradiated T lymphocytes, 15% FCS, and 20% medium conditioned by PHA-stimulated T lymphocytes in 1.0% methylcellulose. Cultures were incubated as described above for 5-7 days, and aggregates of > 20 cells were scored as colonies.

Nonhematologic Tumors

Effects of BA on solid tumors were assessed in vitro using a human tumor colony-forming assay and in vivo using nude mouse xenografts. The human tumor colony-forming assay was performed using the technique of Hamburger and Salmon (10) as previously described (11). Specimens from the malignant effusions or solid tumors of 30 patients were studied (table 1). Only studies in which control cultures grew > 30 colonies/5×10^5 cells were used for analysis. Day 1 colony counts were always < 2/plate. Solid tumors were first minced with scissors, and were then digested for 2 hours with 0.7% collagenase (Collagenase II, Worthington Biochemical, Freehold, NJ). A single-cell suspension was obtained by passing the cells through serially smaller needles, and total cells were plated at 5×10^5 cells/ml. Colonies of > 30 cells were scored after 10-21 days of incubation in 7.5% CO_2.

TABLE 1.—Tumors studied in human tumor colony-forming assays

Tumor type	No. of tumor studies
Primary tumors	
Ovarian carcinoma	15
Epidermoid carcinoma (head and neck)	3
Melanoma	2
Breast carcinoma	2
Mesothelioma	2
Soft tissue sarcoma	3
Neuroblastoma	1
Lymphoma	1
Thyroid carcinoma	1
Xenografts	
Epidermoid carcinoma T222	3
Astrocytoma T24	3

Studies of nude mouse xenografts were performed as previously described (11,12). Tumor CFC were grown from the epidermoid xenograft T222 and astrocytoma xenograft T24, as previously described (11). For in vivo studies, animals were inoculated sc with the T222 tumor or the T380 ovarian carcinoma xenograft. Groups of ten animals were randomized using a table of random numbers when tumors were barely palpable, and were inoculated daily with saline or BA at a dose of 25 or 50 mg/kg. Tumor volume was calculated from measurements of three diameters, as previously described (12), and analyzed according to relative tumor volume (volume on Day x/volume on Day 0, V_x/V_0). After 30 days, animals were killed, and tumor and nontumor body weights were assessed. Histologic sections of tumors were prepared at death using standard techniques, and were stained with hematoxylin and eosin.

RESULTS

The effects of BA on growth of normal and malignant hematologic CFC are shown in figure 1. Dose-dependent inhibition of normal CFU-GM growth was seen over a concentration range of 10-100 μg/ml. Points represent the means ± SE of triplicate experiments, and standard errors were ⩽ 10%. Similar studies of HL60 promyelocytic leukemia colonies also showed dose-dependent inhibition, and the curve was steeper than that observed for normal cells, suggesting selectivity of the compound for normal versus abnormal cells. BA had no significant inhibitory effect on growth of KG-1 cells in colony culture or CFC from two patients with CLL. Results for cell lines also represent the means of triplicate experiments, and SE were again < 10%.

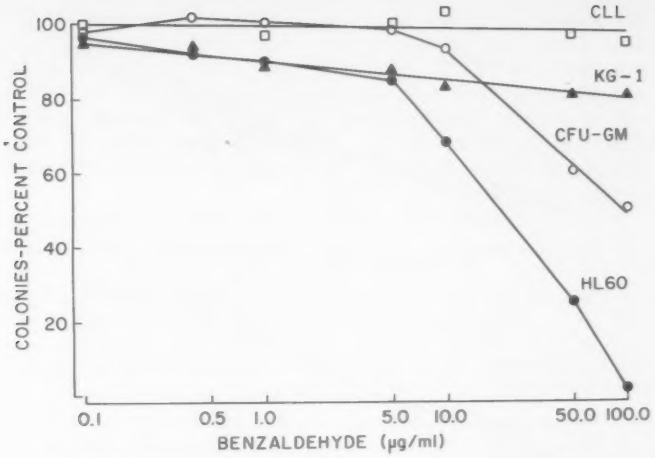

FIGURE 1.—Effects of BA on growth of normal and malignant hematologic CFC.

Because colony-forming assays suggested activity for BA against leukemia cells, its effect on growth of HL60 and KG-1 cells in liquid culture was also assessed. BA failed to inhibit growth of either cell line in liquid cultures (data not shown), and HL60 cells exposed to doses of 50 or 100 μg/ml did not undergo morphologic differentiation, while cells exposed to 1.25% DMSO did undergo differentiation. Thus, although BA showed inhibition of HL60 cells in colony culture, other assessments of its effects on abnormal hematologic cells failed to show significant activity.

Results of human tumor colony-forming assays on 30 patients with various malignancies are shown in figure 2. Dose-dependent inhibition of CFC from several tumors was seen. CFC from three patients with carcinoma of the ovary, and one each with neuroblastoma, mesothelioma, and breast cancer, showed a > 70% reduction in colony growth, a level of inhibition sug-

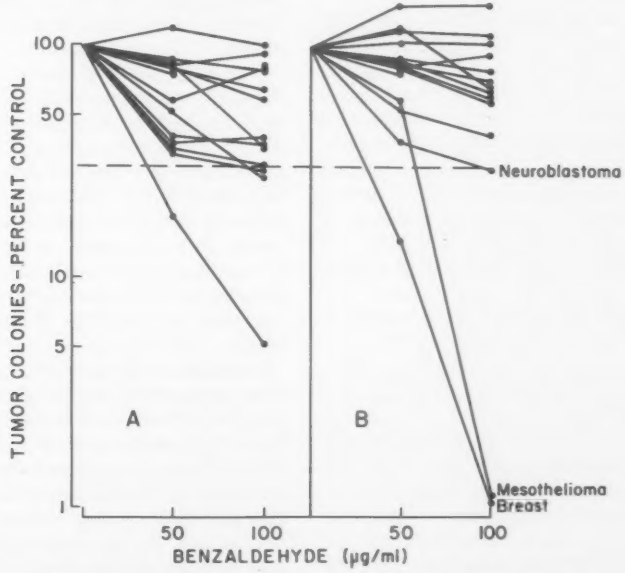

FIGURE 2.—Results of human tumor colony-forming assays on 30 patients.

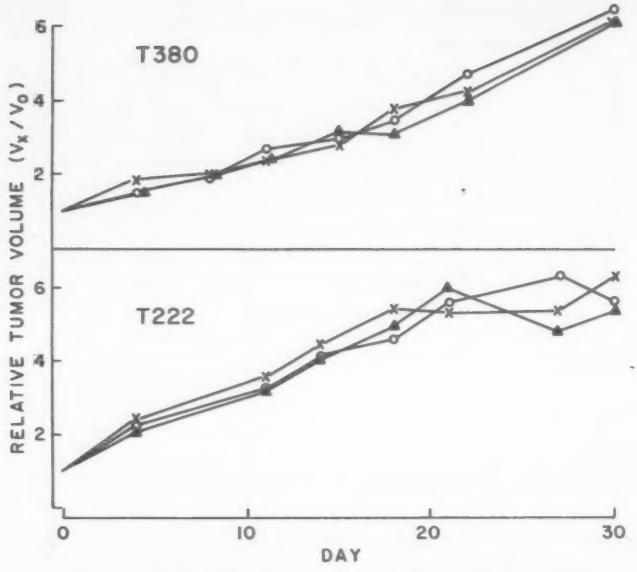

FIGURE 3.—Effects of BA on in vivo growth of the T380 ovarian carcinoma xenograft and the T222 epidermoid carcinoma xenograft.

gested by several groups as dividing sensitive from insensitive tumors (3,13). BA failed to inhibit growth of tumor colonies from either the T222 epidermoid xenograft or the T24 astrocytoma xenograft (data not shown).

The effects of BA on in vivo growth of the T380 ovarian carcinoma xenograft and the T222 xenograft are shown in figure 3. No growth inhibition of either tumor was observed at BA doses of 25 or 50 mg/kg. At death on Day 30, BA and control animals had comparable tumor and nontumor weights. Histologic assessment of the T222 tumors from treated and control groups showed no evidence of keratin "pearl" formation or other evidence of differentiation, as suggested by in vivo studies reported with this drug (6). Therefore, BA failed to show any activity in vivo when tested against these two xenografts, and did not appear to exert nonspecific effects manifest as increased host, nontumor weight.

DISCUSSION

BA is a compound extracted from figs and is proposed as a possible chemotherapeutic agent (14). BA and other related aliphatic aldehydes have been reported to selectively inhibit in vitro growth of SV-40 transformed cell lines (15) and in vivo growth of murine Ehrlich solid tumors (4). The compound has been shown to bind to cell membranes (15,16) and to inhibit uptake of nutrients into SV-40 transformed cells, but not normal counterparts (15). Recently, Kochi et al (6) reported that in the form of β-cyclodextrin, BA induced 33% complete and 18% partial remissions in 57 heavily pretreated patients with advanced solid tumors. Perhaps more important, the compound was without significant clinical toxicity.

Because BA is a natural and nontoxic compound, these reports of antitumor activity were provocative, since the NCI had already declared the compound to be without significant antitumor activity either as BA or cyclodextrin BA. The screening procedures used by the NCI included the following cell lines: in vivo murine leukemia, melanoma, lung, colon, and mammary carcinoma. The compounds were also inactive against the CX-1 colon xenograft implanted as a subrenal capsular implant, and BA was inactive against the CX-1 lung xenograft system (M. K. Wolpert, personal communication).

Recently, several new procedures have become available as potential preclinical screening procedures for new drugs. These include colony-forming assays for malignant hematologic tissues and cell lines, including assays for acute myelogenous leukemia (14), CLL (9), and lymphoma CFC (17). Hamburger and Salmon (10) have also developed a colony-forming assay for human solid tumors, and this system is under evaluation as a possible preclinical screen for new antitumor agents (2,3). Xenografts may also be established from primary tu-

mors, rather than from cell lines such as LX-1 and CX-1, and can be used as in vivo models for screening new drugs and as a source of tissue for human tumor colony growth (18). At various times, we have employed all these techniques to screen new agents (12,19). In addition, we have used an assay for normal marrow progenitors (CFU-GM) to assess selectivity of new drugs (12,19).

We employed these new techniques to reassess activity of BA against human cancer. The compound showed some activity at high doses (10–100 μg/ml) against HL60 promyelocytic leukemia cells grown in colony culture. However, it showed no activity when tested against HL60 cells in liquid culture or against other leukemia cells in colony assays. Disparities between effects of drugs on cell lines in liquid and colony assays have been previously reported (20), and it has been suggested that colony assays are more reliable for drug screening. Although the results with HL60 are intriguing, the weight of the evidence suggests that BA may not have activity against human leukemias.

When the human tumor colony assay was used to assess activity of BA, a > 70% reduction in colony growth was seen in 20% of the patients. A 70% reduction in CFC in vitro has been suggested as identifying patients likely to respond to a drug in vivo, if the drug concentration tested is relevant (3,13). We have found this level of in vitro activity reliable for predicting in vivo activity in xenograft systems if pharmacology of the drug tested was known, but of questionable value when such data were not available (12). The false-positive rate of human tumor colony assay systems for primary tumors is reported to be 30%–58% (13,21). Thus, a maximum of 10%–15% of tested patients would be expected to be responsive to BA, if a concentration of 100 μg/ml could be obtained in vivo. Based on results with this assay system, BA would be expected to lack activity against ⩾ 90% of human solid tumors.

In vitro systems cannot detect active drug metabolites, and drug concentrations chosen for study are always somewhat arbitrary (22). Xenograft systems avoid these shortcomings when tolerable drug doses are employed. When BA was tested against two human xenografts established from primary tumors, no toxicity or antitumor activity was observed. Although the dose tested did not approach toxic levels, the NCI tested toxic levels of BA in vivo and found the compound to be inactive. Further doses reported to induce responses in clinical trials (6) are much lower on a weight/weight basis than those used in vivo in these studies. Further, epidermoid differentiation, reportedly observed in patients with primary tumors, was not observed with the T222 xenograft. Therefore, this system confirmed the lack of activity of BA observed in vitro.

Although the false-negative rate of the NCI drug screening panels remains unknown, little or no evidence has emerged to suggest that active compounds are not detected. Our results, using newer drug screening techniques, are consistent with the ability of the present system to exclude inactive drugs. The high false-positive rate of the drug screen remains a problem. Use of the techniques employed here, and simultaneous comparisons of drug effects on relevant normal tissues such as CFU-GM, may serve to improve the system in the future.

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Efficacy of "Two-Route Chemotherapy" Using Intra-arterial Cisplatin and Iv Sodium Thiosulfate, Its Antidote, in Rat Bladder Tumor[1,2]

Kazuyuki Sagiyama, Jiro Uozumi, Ken Aoki, and Tsuneo Baba[3,4,*]

The efficacy of "two-route chemotherapy (TRC)" using a combination of cisplatin (DDP) and its antidote, sodium thiosulfate (STS), was evaluated in rat bladder tumor. TRC in which 20 mg/kg of DDP and two doses of 1054 mg/kg of STS (100-fold molar ratio to DDP) were given during interruption of arterial flow for 30 minutes through the abdominal aorta and femoral vein, respectively, provided significantly better antitumor effects evaluated by tumor weight and survival time than did intra-arterial or iv DDP (4 mg/kg) without STS. These three modalities revealed a similar toxicity in rats. Dose- and time-related efficacy of DDP and a high sensitivity of a transitional cell carcinoma of the rat to DDP were noted in TRC. Nephrotoxicity assessed by increase in BUN levels was minimum. The particular combination of intra-arterial DDP and iv STS presented here is applicable to regionally confined human bladder cancer. [Cancer Treat Rep 67:567–572, 1983]

The regional arterial infusion of anticancer agents has been widely prescribed since Klopp et al (1) reported its efficacy. The dose to be given, however, is still limited because of unavoidable side effects after the drug enters the systemic circulation.

An improvement in therapeutic efficacy of intra-arterial chemotherapy in rat liver tumors has been achieved in our laboratory by means of a combination of an anticancer agent, mechlorethamine *N*-oxide, and its antidote, cysteine. These drugs were given through the hepatic artery and the tail vein, respectively. The therapy reduced the adverse effects and consequently allowed for an increase in the dose of the anticancer agents. The combination treatment was named "two-route chemotherapy (TRC)" (2).

Recent studies (3,4) showed a potent antagonizing action of sodium thiosulfate (STS) against toxic effects of cisplatin (DDP), which possesses potent anticancer activities against a variety of experimental and human tumors (5–8).

We now report the effectiveness of TRC using a combination of DDP and STS in the bladder tumor. Intra-arterial DDP and iv STS were given during interruption of the regional arterial flow.

MATERIALS AND METHODS

Wistar King A (WKA) female rats weighing 135–165 g at 6–7 weeks of age were provided a standard laboratory diet and tap water ad libitum.

A hepatocellular carcinoma (NH-hepatoma) and a transitional cell carcinoma (RBT-1) induced in WKA rats with 3'methyl-4-(dimethylamino) azobenzene and with *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine, respectively, have been maintained by serial transplantation in the hind limb of syngeneic WKA rats. The single-cell suspension of these tumors was prepared by mechanical dissociation for NH-hepatoma or by trypsin enzymatic dissociation for RBT-1, according to Soloway (9). After a viable cell count with the trypan blue exclusion test, the bladder of a rat was exposed by laparotomy, and 0.025 ml of the cell suspension containing 1.5×10^5 cells of NH-hepatoma or 1×10^6 cells of RBT-1 was injected into the anterior bladder wall.

DDP was provided by Nippon Kayaku Co, Ltd, Tokyo, Japan, and STS was obtained from Wako Pure Chemical Industries, Ltd, Osaka, Japan. DDP and STS were each dissolved in 0.9% NaCl solution at desired concentrations.

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Rats were anesthetized with ether, the lower abdomen was explored, and the following vascular manipulations were carried out under an operation microscope. The abdominal aorta was restricted with 6-0 nylon, 1.5 cm proximal to the bifurcation; a fine needle (27G) with polyethylene tube was inserted into the lumen of the aorta just distal to the restriction and fixed with 8-0 nylon. To minimize outflow of DDP solution to extrapelvic spaces, the uterine arteries, the sigmoidal artery, and the bilateral femoral arteries were clamped during chemotherapy. Then DDP solution in a volume of 2 ml/100 g of body weight was infused into the aorta through the needle using an infusion pump at a flow rate of 4.1 ml/min. STS or saline in a volume of 1 ml/100 g of body weight was given twice, immediately after clamping and just before releasing the restriction of the aorta, via the femoral vein manually at a flow rate of approximately 1 ml/min. After the drug infusions, all the arterial restrictions were released, and bleeding was controlled by a quick-set adhesive. The schematic representation of timing and routes of administration is shown in figure 1. Laparotomy was also carried out in rats given iv DDP treatment and in the untreated controls. DDP was administered iv in a volume of 4 ml/100 g of body weight.

Antitumor efficacy was evaluated by the regression of tumor growth quantitated by tumor weight 14 or 17 days after the treatment or by the prolongation of survival time of rats bearing bladder tumor. Side effects were evaluated by measurement of body weight and BUN levels on various days after treatment. BUN levels in blood samples taken from the tail vein were measured by the urease technique (10).

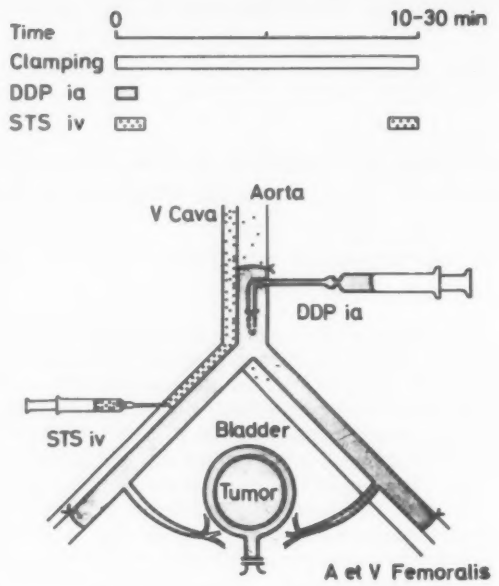

FIGURE 1.—Timing and routes of administration of DDP and STS. ia = intra-arterial.

RESULTS

Table 1 shows the dose-related therapeutic efficacy of DDP by our TRC in rat bladder tumor 6 days after inoculation of NH-cells. The dose of 20 mg/kg of DDP (2.7 × LD50) showed the best antitumor effect when evaluated by the tumor weights 14 days after treatment (Student's t test). No significant changes in BUN levels measured on Day 5 after treatment were detected in any rat given three different doses of DDP.

The effect of duration of arterial flow interruption in TRC on bladder tumor growth was also studied (table 2). It is clear that prolongation of the interruption time tended to provide a better antitumor efficacy, and the best antitumor effect was obtained by interruption for 30 mins. The decrease of mean tumor weight in the 30-min interruption group was statistically significant compared with those in the 20- and 10-min interruption groups ($0.05 > P > 0.01$). The 30-min interruption group without DDP had no antitumor effect.

From the results of dose- and time-related efficacy, we concluded that 20 mg/kg of DDP with 30-min interruption was the best schedule to produce excellent antitumor effects. This dose schedule for TRC was similar in toxicity to intra-arterial or iv DDP at a dose of 4 mg/kg as evaluated by body weight loss. Under such conditions, effectiveness of three modalities of DDP treatment was studied, and figure 2 shows the change in body weight of NH-hepatoma-bearing rats after these treatments. Antitumor efficacy of the treatments was assessed initially by tumor weight of rats treated on Day 2 after tumor inoculation. Mean tumor weights measured 14 days after treatment were 0.07 ± 0.03 g, 0.65 ± 0.11 g, 0.89 ± 0.07 g, and 1.35 ± 0.14 g (n = 8) for rats given intra-arterial DDP at 20 mg/kg with iv STS, intra-arterial DDP at 4 mg/kg, iv DDP at 4 mg/kg, and untreated control, respectively. The mean tumor weight of rats given both DDP and STS by TRC is significantly lower than that of rats given intra-arterial or iv infusion of DDP without STS ($0.05 > P > 0.01$); in the group given TRC, no tumors were visible in the bladders of two of eight rats examined.

The efficacy of various treatments was also evaluated by survival time of rats bearing bladder tumor (NH-cells); the results are summarized in table 3. A greater prolongation of survival time was obtained in the groups of rats given TRC, on Day 2 or on Day 6 after inoculation. The percent increase in lifespan (% ILS) was greater with TRC given on Day 2 than with treatment given on Day 6, indicating that treatment at the earlier stage of tumor growth is more productive. For the rats treated by intra-

TABLE 1.—Effect of DDP dose on NH-hepatoma growth in the bladder and BUN level in TRC*

DDP dose (mg/kg)	No. of rats	Mean tumor weight (g ± SEM)	*P* value†	BUN (mg/dl ± SEM)
Untreated control	6	2.38 ± 0.54		16.9 ± 0.9
5	6	0.82 ± 0.12	< 0.1 (*a*)	20.0 ± 3.1
10	6	0.59 ± 0.22	< 0.05 (*b*)	16.8 ± 1.2
20	6	0.23 ± 0.09	< 0.02 (*c*)	17.0 ± 0.8

*Intra-arterial infusion of DDP at indicated doses with restriction of abdominal aorta for 10 mins and iv STS in 2 doses of 100-fold molar ratio to each DDP dose were given 6 days after inoculation of 1.5×10^5 NH-cells into the bladder wall. BUN level was determined 5 days after treatment.

†Probability was evaluated by Student's t test; (*c*) vs (*a*) is $0.1 > P > 0.05$, and (*c*) vs (*b*) is not significant.

TABLE 2.—Effect of duration of arterial flow interruption in TRC on NH-hepatoma growth in the bladder*

Interruption (mins)	No. of rats	Mean tumor weight (g ± SEM)	*P* value†
Untreated control	6	2.06 ± 0.50	
10	6	0.23 ± 0.09	< 0.02 (*a*)
20	6	0.17 ± 0.05	< 0.01 (*b*)
30	6	0.04 ± 0.02	< 0.01 (*c*)
30 (interruption alone)	6	1.92 ± 0.17	Not significant

*Experimental procedures were similar to those described in table 1, except that periods for arterial interruption were changed as indicated, where DDP and STS were fixed to 20 and 1054 mg/kg, respectively.

†Probability was evaluated by Student's t test: (*c*) vs (*b*) and (*a*) is $0.05 > P > 0.01$.

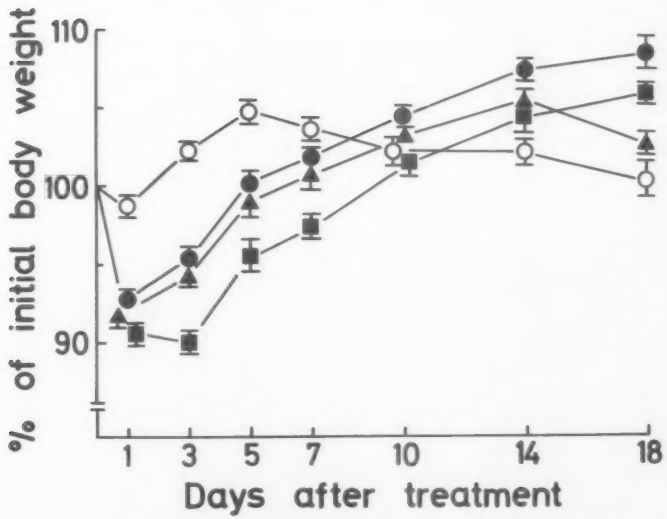

FIGURE 2.—Changes in body weight after various treatments (on Day 6 after inoculation of NH-hepatoma) with DDP alone or DDP + STS. Each value represents mean weight relative to Day 0 ± SEM in 10 rats. ○ = tumor-bearing control; ● = intra-arterial infusion of 20 mg/kg of DDP + STS (1054 mg/kg iv × 2); ■ = intra-arterial infusion of 4 mg/kg of DDP; ▲ = iv infusion of 4 mg/kg of DDP.

TABLE 3.—Effect of various DDP treatments on survival times of rats bearing NH-hepatoma in the bladder

Treatment*	Treatment on Day 2					Treatment on Day 6				
	No. of rats	Survival time (days)		P value†	% ILS‡	No. of rats	Survival time (days)		P value†	% ILS‡
		Range	Median				Range	Median		
Untreated control	10	21-29	26			10	26-32	29		
DDP (20 mg/kg intra-arterially) + STS (1054 mg/kg iv × 2)	10	25-84	44	< 0.01 (*a*)	71	10	25-115	40	< 0.01 (*d*)	38
DDP (4 mg/kg intra-arterially)	10	26-61	32	< 0.01 (*b*)	24	10	28-45	36	< 0.01 (*e*)	24
DDP (4 mg/kg iv)	10	25-35	28	< 0.05 (*c*)	8	10	26-38	31	< 0.05 (*f*)	7

*Treatments were given on Day 2 or Day 6 after inoculation of 1.5×10^5 NH-cells into the bladder wall. Intra-arterial infusion of DDP was given with the restriction of abdominal aorta for 30 mins.

†Probability was calculated by Wilcoxon rank-sum test. (*a*) vs (*b*), $0.1 > P > 0.05$; (*a*) vs (*c*), $0.01 > P > 0.001$; (*d*) vs (*e*) and (*f*), $0.05 > P > 0.01$; (*a*) vs (*d*), $0.1 > P > 0.05$.

‡% ILS was calculated according to the equation. % ILS = 100(t/c - 1), where t is the median survival time of the treatment group in days and c is the median survival time of the control group.

TABLE 4.—Effects of various DDP treatments on tumor growth of RBT-1

Treatment*	No. of rats	No. of rats with tumor (%)	Mean tumor weight (g ± SEM)
Untreated control	4	4 (100)	0.64 ± 0.09
DDP (20 mg/kg intra-arterially) + STS (1054 mg/kg iv × 2)	4	0	0
DDP (4 mg/kg intra-arterially)	4	3 (75)	0.10 ± 0.10
DDP (4 mg/kg iv)	4	4 (100)	0.04 ± 0.02

*Treatment was given on Day 4 after inoculation of 1×10^6 RBT-1 cells. Bladder tumor weight was estimated 17 days after treatment.

arterial or iv infusion of 4 mg/kg of DDP without STS, no difference in % ILS was noted between the two tumor stages.

Table 4 shows the effects of various treatments using DDP on the growth of a transitional cell carcinoma (RBT-1) in rat bladder. Seventeen days after the treatment, no tumor had developed in any bladder of rats treated by TRC (20 mg/kg of intra-arterial DDP with iv STS); tumors developed in the bladders of all other rats, except for one of the four rats given an intra-arterial infusion of 4 mg/kg of DDP without STS.

Nephrotoxicity, the most serious side effect of DDP, was examined after chemotherapy by measurement of BUN levels in rats bearing bladder tumor (fig 3). Intra-arterial infusion of 4 mg/kg of DDP alone produced an increase of BUN level to 36.7 mg/dl on Day 3 after the treatment. On the contrary, BUN levels of rats treated with a five times higher intra-arterial DDP dose (20 mg/kg) in combination with two doses of iv STS (1054 mg/kg) were suppressed to a normal level of 21.5 mg/dl on Day 3, and were slightly increased to 31.1 mg/dl on Day 10.

No pathohistologic change was observed on Days 1, 3, 7, and 14 after the treatment in the kidneys, in the aorta and iliac arteries, or in the bladder mucosa of rats treated with TRC of 20 mg/kg of DDP and two doses of 1054 mg/kg of STS.

DISCUSSION

The side effects with intra-arterial infusion of DDP are similar to those seen in cases of iv administration (11,12). Although a combination of extensive hydration and diuresis by mannitol has currently been carried out to reduce DDP toxicity, this method is incomplete in protecting against the nephrotoxicity of a large dose of DDP (13). Recently, a thiol compound, STS, has been shown to have a potent antagonistic action against DDP-induced toxicity in mice without any toxic effects in itself, even at high doses (3,4). Formerly, STS had been used in fairly large doses both in cyanide poisoning and in renal function tests (14). The administration of DDP and STS via the same route eventually resulted in a reduction of antitumor activity (3); however, in the present study, we found a remarkable improvement in therapeutic effects

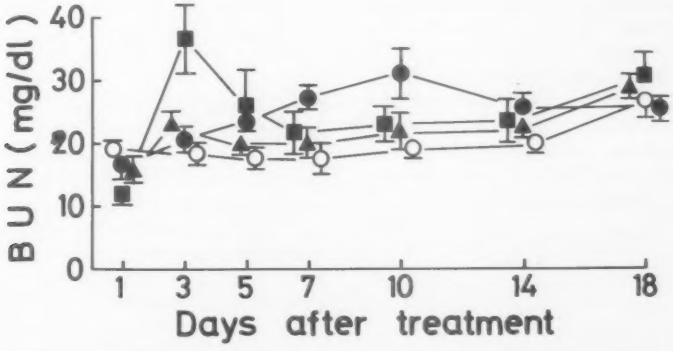

FIGURE 3.—Changes in BUN after various treatments (on Day 6 after inoculation of NH-hepatoma) with DDP alone or DDP plus STS. Each value represents mean BUN (mg/dl ± SEM) in 10 rats. ○ = tumor-bearing control; ● = intra-arterial infusion of 20 mg/kg of DDP + STS (1054 mg/kg iv × 2); ■ = intra-arterial infusion of 4 mg/kg of DDP; ▲ = iv infusion of 4 mg/kg of DDP.

of DDP on rat bladder tumor by giving DDP and STS through different routes.

The restriction of the abdominal aorta was made to avoid flushing out of DDP from the tumor region and to prevent STS from entering the region. The dose- and time-related efficacy of DDP observed with this arterial flow interruption may suggest that a higher local concentration of DDP and a longer contact of DDP with tumor cells were achieved by increasing the dose of intra-arterial DDP and by prolongation of the interruption time, respectively.

We reported that a single administration of STS at a molar ratio of 100-fold to DDP immediately after the arterial restriction failed to protect rats from the lethal toxicity of 20 mg/kg of DDP, but an additional dose of STS about 10 mins after the first administration resulted in complete protection against DDP-induced toxicity (15). In the present study, two STS injections, when given at about 30-min intervals, produced an increase in BUN level to > 30 mg/dl on Day 10 (fig 3). However, this rise of BUN was prevented by giving an additional dose of STS in the middle of the 30-min intervals (data not shown). These results indicate that since a very rapid clearance of STS occurs in vivo (14), the plasma concentration of STS about 15 mins after administration is not sufficient to antagonize DDP leaking out from the regional artery.

Although the hepatocellular carcinoma (NH-hepatoma) used in this study responded well to DDP, a transitional cell carcinoma of the rat (RBT-1) was more sensitive to DDP in the TRC treatment (table 4). Since Soloway (9) showed that there is a correlation of sensitivities to DDP between human and murine transitional cell carcinoma, TRC using DDP and STS may be effective for human bladder cancer.

TRC using DDP and STS was already applied in cases of peritoneally disseminated cancer and metastatic liver tumors in rats, with high therapeutic efficacy, in our laboratory (16,17). Its applicability for regionally confined human cancers shows great promise.

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Multidrug Chemotherapy Using Bleomycin, Methotrexate, and Cisplatin Alone or Combined With Radiotherapy in Advanced Head and Neck Cancer[1]

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Twenty-two patients with advanced cancer of the head and neck where treated with a combination of bleomycin, methotrexate, and cisplatin. In ten previously untreated patients, a complete response (CR) rate of 20%, a partial response rate of 50%, and a no change rate of 30% were observed. With the addition of radiotherapy, the local CR rate in this group of patients increased to 60%. In 12 patients with recurrent disease after surgery and/or radiotherapy, the CR rate was 25%, the partial response rate was 16.7%, and the no change rate was 58.3%. Toxic effects were acceptable, with no fatalities. These short-term results encourage accrual of more patients in the study and further follow-up to determine whether the cure rate can be increased by using cisplatin and radiotherapy in patients with advanced previously untreated head and neck cancer. More energetic treatment should be investigated in patients having disease recurrence after radiotherapy. [Cancer Treat Rep 67:573–574, 1983]

Used as a single-treatment modality or in combination, surgery and radiotherapy can control only 15% of advanced head and neck cancer (1). Combination chemotherapy using bleomycin, methotrexate, and cisplatin (BMP) has been shown to be effective in advanced disease by Kaplan et al (2) and Vogl et al (3), who achieved a response rate of 63%. In this paper, we report our experience with combination chemotherapy using BMP followed by radiotherapy in advanced previously untreated head and neck cancer and our results with combination chemotherapy in surgery and/or radiotherapy failure.

MATERIALS AND METHODS

Between May 1980 and August 1981, 25 patients with advanced or recurrent head and neck cancer received combination chemotherapy using BMP. Three patients with previously untreated disease were omitted from this report: one had non-squamous cell cancer; and two, who started receiving chemotherapy and showed response, refused further treatment using radiotherapy according to the protocol and were lost to follow-up. Among the 22 evaluable patients, there were 17 males and five females, with a mean age of 52 years. Ten patients (Group A) had advanced stage III–IV previously untreated cancer, considered inoperable, and were to receive three courses of combination chemotherapy, to be followed 3 weeks after completion of the third course by radiotherapy.

Twelve patients (Group B) received the drug combination after local-regional surgery and/or radiation therapy failure. One patient had distant metastases in addition to local disease recurrence. In this group of 12 patients, chemotherapy was continued until disease progression.

Chemotherapy was administered according to the regimen reported by Kaplan et al (2), and consisted of bleomycin (10 units im on Days 1, 7, and 15 of each course), methotrexate (40 mg/m^2 im on Days 1 and 15 of each course), and cisplatin (50 mg/m^2 by iv infusion on Day 4). This regimen was repeated every 21 days.

Radiotherapy was given through combined cobalt-60 photon and 8-mev electron beams. Treatment fields included the primary tumor and the entire neck. Involved sites received a dose of 6000–7500 rads in 6–7½ weeks. Uninvolved lymph node areas received 5000 rads in 5 weeks.

RESULTS

The ten Group A patients had the following response rates after three courses of BMP: two patients (20%) had

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TABLE 1.—Results of BMP alone or followed by radiotherapy in advanced head and neck cancer

	Group A (BMP + radiotherapy)	Group B (BMP)
No. of patients	10	12
Response rate*		
CR	60%	25%
PR	40%	16.7%
No change	—	58.3%
No. of responses according to site (CR/PR)		
Larynx	1/1	1/3
Nasopharynx	6/6	1/2
Oral cavity	1/1	2/4
Parotis (squamous cell carcinoma)	—	1/2
Maxillary sinus	1/1	—
Cervical lymph nodes (unknown origin)	1/1	0/1

*Duration of response in mos: Group A—10+, 10+, 11+, 12+, 15+, and 21+ (CR); Group A—3, 11+, 11+, and 12+ (PR); Group B—4, 7, and 7 (CR); and Group B—4 and 9 (PR).

complete remission (CR), five (50%) had partial remission (PR), and three (30%) had no change. Three of the five patients who achieved PR with chemotherapy achieved complete local control after radiotherapy. Two patients with no change after chemotherapy achieved PR after radiotherapy. One patient with no change had complete control of his local disease by radiation therapy, but later developed bone metastases. Thus, six of ten patients in Group A (60%), who completed the whole treatment plan consisting of three courses of BMP followed by radiotherapy, achieved local CR and four patients (40%) achieved PR. Duration of response ranged between 3+ and 16+ months and 10+ and 21+ months, respectively, in partial and complete responders.

The 12 Group B patients had the following response rates after a minimum of three courses of BMP: three patients (25%) had CR, two (16.7%) had PR, and seven (58.3%) had no change. Remission was maintained between 4 and 9 months. Results are summarized in table 1.

The toxic effects of chemotherapy were acceptable, with no fatalities. Only three patients (13.5%) had a wbc count $< 2000/mm^3$, and only one patient (4.5%) had a platelet count $< 75,000/mm^3$. Septicemia occurred in one patient (4.5%). There were no cases of nephrotoxicity or pulmonary toxicity.

DISCUSSION

The number of reported studies on the effect of cisplatin-containing chemotherapy regimens followed by radiotherapy in advanced head and neck cancer is limited (4,5). Vogl et al (5) showed that the combination of BMP followed by radiotherapy or surgery resulted in improved initial control rates, but with no benefit to long-term survival of patients. In contrast to these data, our present results indicate that with the same drug combination followed by radiotherapy, better local control rates may be achieved in advanced head and neck cancer.

The discrepancy between our results and those of Vogl et al (5) can be attributed to the fact that most of Vogl's patients had oral cavity cancer, while most of our patients had nasopharynx carcinoma. This observation deserves further investigation.

According to our data, the value of BMP in surgery and/or radiotherapy failure is limited, and a more vigorous treatment protocol should be explored.

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Vincristine in Advanced Bladder Cancer: A European Organization for Research on Treatment of Cancer (EORTC) Phase II Study[1,2]

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Thirty-seven patients with measurable deposits or primary lesions arising from transitional cell carcinoma of the bladder were treated with 1 mg/m²/week of vincristine. One complete response lasting 7 months was seen among the 23 patients with metastatic disease. Two partial responses were seen among the 14 patients with persistent primary tumor. Vincristine is not recommended for further evaluation as a single agent in transitional cell carcinoma of the bladder. [Cancer Treat Rep 67:575–577, 1983]

Vincristine (VCR) is an alkaloid extracted from *Vinca rosea*, which is active against leukemias, lymphomas, and a variety of solid tumors (1). When tested against transplantable bladder carcinoma, it was found to be as active as doxorubicin and bleomycin and more active than alkylating agents such as cyclophosphamide and mitomycin (2).

There has been little evaluation in human bladder cancer, although remissions were reported in three of five patients with transitional cell carcinoma of the bladder who had been judged, prior to treatment, to have an estimated survival of at least 2 months (1). Since this response rate appeared comparable to the 35%–40% rate obtained with cytostatic agents such as cisplatin (3,4) and methotrexate (5,6), we decided to evaluate VCR using a larger sample.

This EORTC Urological Group study describes the antitumor activity of VCR tested in patients with measurable primary or metastatic deposits of transitional cell carcinoma of the bladder.

MATERIALS AND METHODS

Forty-three patients were entered in the study. Six patients were ineligible (inadequate performance status, three patients; no biopsy, one; and radiotherapy to the indicator lesion, two). The remaining 37 patients had measurable primary or metastatic deposits arising from a biopsy-proven transitional cell carcinoma of the bladder, with an estimated survival of > 90 days and an adequate performance status (World Health Organization [WHO], 0–2) (7). No patient had received: (*a*) prior chemotherapy within 1 month of entry in the study; (*b*) radiotherapy involving the indicator lesion within 3 months; or (*c*) treatment with vinca alkaloids at any time.

Bidimensionally measurable metastatic disease was seen in 23 patients (lung, 12 patients; lymph nodes, seven; subcutaneous tissue, two; or bone [osteolytic deposits], two). An additional 14 patients, analyzed separately, had measurable primary disease of the bladder. VCR was given iv at a dose of 1 mg/m²/week (maximum, 2 mg) for an induction course of 8 weeks. For patients

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who did not have disease progression after the 8-week induction course, VCR was continued at the same dose every 2 weeks.

Response Criteria

Complete response (CR) was defined as the complete disappearance of all known disease, confirmed by two observations at least 1 month apart. Partial response (PR) was defined as a reduction of 50% in the summed products of the two largest perpendicular diameters of all indicator lesions, with no new lesions appearing. Progressive disease was defined as the appearance of any new lesion, or any existing lesion increasing by > 25% of the product of the two largest perpendicular diameters. No change was defined as any changes in the indicator lesions that do not fulfill the criteria for disease progression or response.

Toxicity Criteria

Hematologic toxicity was evaluated according to WHO criteria (7).

Neurologic toxicity was evaluated as follows: *grade 1 (mild)*—tolerable paresthesia, loss of tendon reflexes, constipation, or mild gastrointestinal upset; *grade 2 (moderate)*—painful paresthesia, sensory loss, disabling weakness, abdominal cramps, or moderate gastrointestinal upset; and *grade 3 (severe)*—paraplegia or gastrointestinal upsets severe enough to require cessation of therapy.

RESULTS

Thirty-seven eligible patients were entered from 13 institutions in six countries. Of the 23 patients with measurable metastatic disease, 14 showed disease progression at the 8-week assessment, eight showed no change, and one had a PR of pulmonary metastasis. This patient's pulmonary deposit continued to decrease in size and a CR was achieved at 16 weeks, by which time the patient had refused further treatment with VCR because of abdominal cramps. The CR lasted for 7 months. Four of these 23 patients received fewer than four doses of VCR. Twelve of the 14 patients with measurable primary tumors received at least four courses of VCR. Ten patients had disease progression, two showed no change at the 8-week assessment, and two had a PR lasting 8 and 14 weeks, respectively. Considerable neurotoxicity was noted, and is shown in table 1. None of the responding patients had had prior chemotherapy.

DISCUSSION

Holland et al (1) noted three PR in five patients given VCR at the dose of at least 50 μg/kg/week (approximately 2 mg/m²/week, twice that used in the present study). In their broad phase II study, a progressive dose reduction was introduced without decreasing the response rate, probably because there was less toxicity at lower doses and more courses of therapy could be given. The small dose used in the present study was still associated with significant side effects.

Only one response (CR for 28 weeks) was seen in 23 patients with measurable metastatic disease. If those patients with persistent cancer of the bladder (a group in which the extent of the disease is hard to evaluate) are included, the response rate is 8% (three responses among 37 patients). Response rates of 35%–40% have been achieved with cisplatin alone (3), in combination (4), and with methotrexate (5,6). The chance that a true response rate of 30% is concealed in the rates obtained in the present study is slim. If the calculation includes all 37 eligible patients, the 95% confidence interval for the true underlying response rate is 2%–22% and the probability of observing three or fewer responses is only $P = 0.001$ if the true response rate is 30%.

This analysis includes all eligible patients, whether or not they actually received an adequate course of treatment. Six patients (four in the metastases group and two in the primary group) had treatment which was possibly inadequate, with four dying of rapid progression before they had received four courses of VCR, and one refusing

TABLE 1.—Patient characteristics

Characteristic	Group	
	Metastases	Primary
Total No. of patients	23	14
Males/females	21/2	12/2
Age (yrs)		
Range	54-77	51-78
Median	66	65
Previous treatment (No. of patients)		
Irradiation to bladder	13	13
Chemotherapy (cisplatin, cyclophosphamide, and doxorubicin)	12	8
Response (No. of patients)		
CR	1	0
PR	0	2
No change	8	2
Disease progression*	14	10
Toxic effect (No. of patients)†		
Bone marrow	0	
Neurologic		
None	14	
Mild	12	
Moderate	6	
Severe	3	
Nausea	2	

*Includes 5 patients (3 in the metastases group) who died of disease within 4 wks and 1 (in the metastases group) who refused further treatment after 1 course.

†Includes both groups.

treatment after one course. If these six patients are excluded from the analysis, the response rate becomes one among 19 patients in the metastases group, two among 12 in the primary group, and three among 31 in both groups combined, with the 95% confidence interval ranging from 2%–26%. The probability of observing three or fewer responses in 31 patients if the true response rate is 30% remains low at $P = 0.007$.

Further evaluation of VCR as a single agent in transitional cell carcinoma of the bladder is not recommended.

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Phase I Evaluation of Semustine in a Weekly Schedule[1]

Charles A. Koller,* Darlene Schwalm, Marcia Liepman, Ronald B. Natale, and Richard H. Wheeler[2]

A phase I study of weekly semustine was conducted in 32 patients with advanced cancer. Doses were escalated from 18 to 36 mg/m²/week. At a dose of 36 mg/m²/week, 43% of the patients developed platelet counts < 100,000/mm³ after a median of 20 weeks on study, and 25% developed wbc counts < 3000/mm³ after a median of 16 weeks on study. Drug-related nausea was reported by only one patient and was the only subjective toxic effect reported. Tumor regressions were noted in patients with adenocarcinoma of the colon, melanoma, and mixed adenocarcinoma and squamous cell lung cancer. A reasonable starting dose for phase II studies is 36 mg/m²/week, with dose adjustments based on platelet and wbc counts. [Cancer Treat Rep 67:579–581, 1983]

Semustine is a nitrosourea compound which has shown significant antitumor activity against a wide variety of animal tumor model systems and human solid malignancies and lymphomas (1). Unfortunately, nitrosoureas also possess delayed and cumulative myelosuppression which, along with severe nausea and vomiting, limits the frequency and total dose of drug that can be administered (2). Thus, available nitrosoureas have enjoyed only limited success in cancer therapy to date. Since the antineoplastic effects of nitrosoureas may be due to different mechanisms than the toxic effects (3), preclinical studies were devised to improve the therapeutic index of semustine. For example, a murine Lewis lung carcinoma model showed definite schedule dependence with improved responsiveness and reduced toxicity when two smaller doses of semustine were administered at 8–16-day intervals compared to results obtained from a single, higher dose (4). Therefore, a phase I study was undertaken to assess weekly low-dose oral semustine because of the above-mentioned superior therapeutic index in the murine system.

MATERIALS AND METHODS

All patients participating in this study had histologic proof of malignancy not curable by surgery, radiotherapy, and/or established standard chemotherapy, and had an estimated life expectancy of at least 8 weeks. A summary of previous chemotherapy and radiation therapy is shown in table 1. Patients had received no chemotherapy or radiation therapy in the 4 weeks prior to this study. All patients had a wbc count > 3000/mm³, a platelet count > 150,000/mm³, a hemoglobin level > 10 g/dl, and adequate renal (serum creatinine < 2.0 mg/dl) and hepatic (serum bilirubin < 3.0 mg/dl) functions. All patients were ambulatory and were maintained on oral nutrition.

There were 13 patients with colon cancer; eight with lung cancer (four with adenocarcinoma, three with squamous cell carcinoma, and one with mixed adenocarcinoma and squamous cell carcinoma); six with malignant melanoma; and one each with renal cell carcinoma, malignant carcinoid, and adenocarcinomas of the esophagus, stomach, and pancreas. Three patients had dose escalations. No patient had received prior nitrosoureas.

Pretreatment evaluation included a medical history and physical examination, weight, performance status, tumor measurement (if possible), chest roentgenogram, ECG, cbc with differential, platelet count, urinalysis, serum BUN, creatinine, uric acid, total and direct bilirubin, SGOT, SGPT, and alkaline phosphatase. Cbcs with differential and platelet count were done weekly. Serum chemistry tests were repeated every 4 weeks along with clinical evaluation of disease response.

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TABLE 1.—Weekly semustine dose levels vs toxicity

Dose (mg/m²)	No. of patients entered/ evaluable	Prior treatment (radiation therapy/ chemotherapy/none)	Median No. of doses (range)	Leukopenia < 3000 cells/mm³			Thrombocytopenia < 100,000 cells/mm³		
				No. of patients	Median wks to onset (range)	Median wbc count nadir (range)	No. of patients	Median wks to onset (range)	Median platelet count nadir (range)
18	3/2	1/2/0	6 (2-8)	—	—	5.9 (5.7-6.1)	—	—	347 (302-393)
24	4/4	3/1/1	11 (11-12)	—	—	4.3 (3.4-5.5)	—	—	192 (146-230)
30	11/7	4/5/3	6 (4-26)	—	—	5.0 (3.1-9.2)	1	7	175 (89-235)
36	17/16	4/7/5	12 (6-50)	4	16 (10-60)	3.8 (2.5-6.8)	7	20 (6-58)	108 (19-219)

tute (Bethesda, MD) through the Ohio State University Comprehensive Cancer Center in 10- and 50-mg capsules which were kept frozen at -20°C until dispensed. The starting dose was 18 mg/m²/week on this protocol. Dose escalations are shown in table 1 and were carried out if no toxicity was observed at the lower dose level. Therapy was temporarily withheld when the weekly blood cell count showed wbcs < 3000/mm³ or platelets < 100,000/mm³ and were reinstated when the blood cell counts exceeded these levels.

RESULTS

A total of 35 patient trials were entered in the study; 29 were evaluable for toxicity. Three of six patients were not evaluable for toxicity because of evidence of progressive disease after < 4 weeks in the study; three patients moved and were lost to follow-up. Patient compliance with this study was high. No patient declined further therapy because of subjective toxicity. Nausea was reported by three patients; it was related to progressive disease in two patients (bilateral ureteral obstruction with uremia in one and bowel obstruction in the other).

The dose-limiting toxic effects were thrombocytopenia (< 100,000 cells/mm³) in eight patients and leukopenia (< 3000 cells/mm³) in five. No patient had evidence of hematologic toxicity < 6 weeks from initiation of therapy (table 1). Hematologic nadirs occurred 1–2 weeks after discontinuing semustine in all patients but one. This patient, severely malnourished because of advanced colon cancer, had an asymptomatic platelet count nadir of 19,000/mm³ 3 weeks after discontinuing therapy. She was the only patient in this study who developed a platelet count < 50,000/mm³. One patient with a history of massive hemoptysis prior to therapy had a recurrence of hemoptysis when his platelet count dropped to 55,000/mm³. He was the only patient who required platelet transfusions. There were no episodes of sepsis or unexplained fever in the patients with leukopenia. No renal or hepatic toxic effects were noted in our small cohort.

There were three responses among the 12 patients who completed 8 weeks of study at the 36-mg/m² dose level. One patient with adenocarcinoma of the colon with lung metastases had a complete remission lasting 151 days as evidenced by a normal chest roentgenogram and normalization of the CEA. One patient with mixed adenocarcinoma and squamous cell carcinoma of the lung had a partial response lasting 13 months. One patient with malignant melanoma had disease stabilization lasting 26 months. This patient received a total dose of 3800 mg of semustine and has developed no long-term toxic effects 2 years after discontinuing the drug.

DISCUSSION

Semustine has both subjective and objective toxic effects which limit its use despite impressive antitumor activity in vitro and in animal tumor systems. Using a new regimen of lower doses of semustine at more frequent intervals, we did not observe the severe nausea, vomiting, and malaise that represent well-known toxic effects on the standard dose regimen. Hematologic toxicity was modest and easily manageable. Larger and longer-term studies must be undertaken to assess cumulative myelosuppression, but several patients tolerated high cumulative doses over time. The weekly administration of semustine resulted in tumor regressions in three of the 12 patients receiving 36 mg/m² for > 8 weeks. Thus, phase II trials appear to be indicated. We recommend a starting dose of 36 mg/m² with dose modifications based on platelet and wbc counts in an attempt to administer a weekly dose of semustine that can be given with the fewest possible interruptions.

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Treatment of Metastatic Carcinoid Tumor With Dactinomycin or Dacarbazine[1]

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We studied the effect of two single drugs on patients with metastatic carcinoid tumors. All patients had symptomatic and measurable disease and all had elevations of urinary 5-hydroxy indole acetic acid excretion. Seventeen patients were treated with dactinomycin at a dose of 12–15 μg/kg/day for 5 days repeated every 4–5 weeks. One partial response was achieved. Fifteen patients were treated with dacarbazine (DTIC) at a dose of 250 mg/m²/day for 5 days every 4–5 weeks. Two partial responses were achieved. The median times to tumor progression were 10 weeks with dactinomycin and 18 weeks with DTIC. Median survival times were 28 and 47 weeks, respectively. Dactinomycin given by this schedule appears to have only limited activity as a single-agent treatment in malignant carcinoid tumor. DTIC, although showing only a slightly better response rate, perhaps warrants further investigation in view of our more favorable interval to tumor progression and survival experience and in view of this drug's more established activity in the closely related islet cell carcinoma. [Cancer Treat Rep 67:583–585, 1983]

The data on single-drug chemotherapy in carcinoid tumor are sparse due to the rarity of this tumor. Much of the available information is in the form of single case reports or very small series of cases. Dactinomycin has been reported to show activity in three of five patients (1). Dacarbazine (DTIC) has been reported to show activity against malignant islet cell tumors (2–5). Kessinger et al (6) treated two carcinoid syndrome patients with DTIC and observed objective improvement in one and subjective improvement in the other. We report on two separate prospective studies employing dactinomycin or DTIC as single drugs in the treatment of metastatic carcinoid tumors with the hope that larger patient experiences could place the role of these drugs into a better perspective.

PATIENTS AND METHODS

All patients had histologically proven symptomatic unresectable metastatic carcinoid tumor and all had elevated 5-hydroxy indole acetic acid (5-HIAA) in 24-hour urine studies (normal, < 9 mg/24 hours). No patient was treated with an Eastern Cooperative Oncology Group (ECOG) performance score > 3.[3] All patients had measurable disease defined as areas of tumor clearly measurable across perpendicular diameters on physical examination or chest x-ray, hepatomegaly in which the liver extended at least 5 cm below the xiphisternum or costal margin on quiet respiration, or elevation of urinary 5-HIAA excretion to ≥ 25 mg/24 hours. If a liver scan was employed, a clearly measurable area of decreased uptake at least 5 cm in diameter had to be present. Treatment was deferred for at least 4 weeks following prior chemotherapy, radiation therapy, or surgery. Dactinomycin was given by rapid iv injection at a dose of 12–15 μg/kg/day for 5 days repeated every 4–5 weeks. DTIC was given by rapid iv injection at a dose of 250 mg/m²/day for 5 days every 4–5 weeks. If the 24-hour urinary excretion of 5-HIAA was > 150 mg or a clinically florid carcinoid syndrome was present, the initial dose was reduced by 50%. In subsequent courses the dose was escalated to the higher level as tolerated. If excessive toxicity was encountered with initial treatment, subsequent courses were reduced to levels producing definite but clinically tolerable toxicity. Patients were evaluated every 4–5 weeks with a full clinical examination, measurement of

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[3]ECOG performance status: 0 = fully active; 1 = working part-time; 2 = unable to work but ambulatory > 50% of waking hours; 3 = ambulatory < 50% of waking hours but able to care for self; and 4 = totally disabled.

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indicator lesion, and repetition of 24-hour urinary 5-HIAA determinations. An objective response required a 50% reduction in the product of the perpendicular diameters of measurable indicator lesions or if hepatomegaly was the indicator, a 30% reduction in the sum of the measurements below each costal margin and xiphisternum. 5-HIAA urinary excretion was the indicator only if patients did not have measurable tumor areas. If 5-HIAA was the indicator, it was required that the pretreatment level be > 25 mg/24 hours and that the level be reduced by at least 50%. In all cases no new lesions or increases in nonindicator lesions were allowed. Treatment was continued until tumor progression occurred.

RESULTS

Seventeen patients were treated with dactinomycin and 15 were treated with DTIC. The clinical characteristics of these patients are shown in table 1.

Both drugs produced mild to moderate hematologic toxicity, as expected. Mucositis, dermatitis, and diarrhea were associated with dactinomycin and nausea and vomiting were more severe with DTIC. One patient treated with dactinomycin died of CNS metastases 2 weeks after treatment. One patient treated with DTIC died suddenly of unknown causes 2 weeks after initial treatment.

Objective response occurred in one of 17 patients treated with dactinomycin. The indicator lesion was hepatomegaly, which showed a complete response, and the 24-hour urinary 5-HIAA also showed a marked reduction from 212 to 26 mg in 24 hours. This response has continued for > 7 years. Two patients treated with DTIC responded; both had a > 50% reduction in liver measurements and a reduction in 24-hour urinary 5-HIAA from 140 to 60 mg in 24 hours and from 351 to 201 mg in 24 hours. The responses lasted 4 and 10 months, respectively.

The median time to tumor progression for the patients treated with dactinomycin or DTIC was 10 and 18 weeks, respectively. The median survival for patients treated with dactinomycin or DTIC was 28 and 47 weeks, respectively.

TABLE 1.—Patient characteristics*

Characteristic	Treatment	
	Dactinomycin	DTIC
Sex		
Males	11	6
Females	6	9
Age in yrs		
Median	61	54
Range	43-79	41-67
Primary site		
Small bowel	14 (82%)	8 (53%)
Stomach		1
Lung		1
Unknown	3	5
Urinary 5-HIAA (mg/24 hrs) (pretreatment)		
Median	94	186
Range	17-351	20-324
Prior chemotherapy	14 (82%)	12 (80%)
5-FU	13	8
Streptozocin	11	6
Cyclophosphamide	5	3
Doxorubicin	6	5
Other	6	5
Prior responses		
Complete	1	—
Partial	4	2
ECOG performance score		
0-1	7	5
2-3	10	10
Median time from diagnosis to study treatment (mos)	30.9	21.2

* Unless otherwise specified, values = No. of patients.

DISCUSSION

Responses reported for single drugs in metastatic carcinoid tumor include: 5-FU, five responses among 19 patients (7) and two responses among 11 patients (8); doxorubicin, seven responses among 33 patients (7); mitomycin, one response among four patients (7); streptozocin, one response among six patients (7) and no responses among eight patients (9); cyclophosphamide, four responses among 16 patients (10) and no responses among eight patients (8); methotrexate, one response among six patients (10); and melphalan, two responses among eight patients (10) and no responses among seven (7) patients.

There are few data on combination therapy. A combination of cyclophosphamide and methotrexate was reported to produce responses in six of 11 patients (10). The ECOG randomized 118 patients with metastatic carcinoid tumor to either streptozocin plus 5-FU or streptozocin plus cyclophosphamide (8). Objective response rates were 33% and 26%, respectively. Clearly, more active drugs need to be identified for this disease.

We have investigated the activity of dactinomycin and DTIC in this disease. With only one response in 17 patients, we feel that dactinomycin has limited activity in the dose and schedule that we employed. DTIC with two responses in 15 patients also has limited activity but further studies may be warranted in view of the favorable responses reported by others and in view of the more substantive reports of its activity in the related islet cell carcinoma.

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Cisplatin-Induced Changes in Bleomycin Elimination[1,2]

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Bleomycin disposition was studied in two children who received at least six courses of vinblastine, bleomycin, and cisplatin. Total plasma clearance of bleomycin decreased from 39 to 18 ml/min/m², while the terminal-phase half-life increased from 4.4 to 6.0 hrs since the cumulative cisplatin dose was > 300 mg/m². In one patient, renal clearance of bleomycin decreased from 30 to 8.2 ml/min/m². Changes in serum creatinine or BUN were not predictive of changes in bleomycin elimination. [Cancer Treat Rep 67:587–589, 1983]

The bleomycins are a highly water-soluble group of complex glycopeptides which act as antitumor agents primarily by an excising effect on free bases after binding to DNA. Although the drug does not have appreciable marrow toxicity, it does produce mild nausea and vomiting, fever, and skin and pulmonary toxicity. Bleomycin is used in combination with cisplatin in the treatment of nasopharyngeal carcinoma, ovarian germ cell tumor, and testicular carcinoma in children (1).

Bleomycin is primarily excreted unchanged in the urine, and since cisplatin is a nephrotoxin, we studied the effect of concomitant cisplatin therapy on bleomycin disposition. We present two patients who have delayed bleomycin elimination with long-term cisplatin therapy.

MATERIALS AND METHODS

Patients

The pharmacokinetics of bleomycin were determined after six iv bolus courses administered to two patients. Informed consent was obtained from each patient. Patient 1 was a 14-year-old white female with metastatic nasopharyngeal carcinoma, while Patient 2 was a 16-year-old white female with recurrent ovarian germ cell tumor.

Both patients had normal renal and liver function tests upon entry in the study. Creatinine clearance was estimated with the Schwartz equation (2). Although both patients had received prior chemotherapy, neither patient had received more than one previous course of cisplatin or other known nephrotoxins. Each patient received a combination regimen consisting of vinblastine and bleomycin (30 mg/m², Day 1) and cisplatin (90 mg/m², Day 2) every 3–4 weeks.

Blood and Urine Samples

Blood samples were obtained in heparinized tubes at 5, 15, and 30 mins and 1, 4, 8, 12, and 24 hrs after drug administration. In Patient 2, urine was also collected.

Assay Procedure

Plasma and urine samples were stored at -70°C and were assayed for bleomycin in duplicate by radioimmunoassay (3). This method can measure concentrations as low as 8 ng/ml, and the precision of the assay within a day and from day to day is 8% and 10%, respectively.

Data Analysis

Bleomycin plasma concentration-time data were analyzed by model-dependent and compartment model-independent methods with the DRUGMODEL program (4) available through the PROPHET system. All bleomycin concentration data were weighted as the reciprocal of the measured value (1/Y). This analysis provided

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TABLE 1.—Serial clinical and pharmacokinetic data for Patients 1 and 2

	Course No.					
	Patient 1				Patient 2	
	1	2	3	4	1	2
Cumulative dose (mg/m²)						
Bleomycin	30	120	150	190	0	210
Cisplatin	90	270	360	480	0	630
Bleomycin pharmacokinetics						
β-half-life	3.2	3.2	3.5	4.7	6.8	9.8
CL_T (ml/min/m²)	42.7	45.7	18.7	23.2	30.5	12.3
CL_R (ml/min/m²)	—	—	—	—	30.0	8.2
Renal function tests*						
Serum creatinine (mg/100 ml)	0.8	0.7	1.0	1.1	0.5	1.5
BUN (mg/100 ml)	12.3	18.5	12.7	16.9	8.0	10.0
Estimated creatinine clearance† (ml/min/1.73 m²)	113	130	91	83	173	58
Measured creatinine clearance (ml/min/1.73 m²)	—	94	—	49	—	—

*Normal values: serum creatinine, 0.4–1.3 mg/100 ml; BUN, 8–20 mg/100 ml.
†Estimated by the Schwartz equation (ref 2).

estimates of the rate constants α and β, volume of the central compartment, volume of distribution at steady-state, and total plasma clearance (CL_T). Volume of distribution at steady-state and CL_T were also estimated with model-independent methods. Renal clearance (CL_R) was calculated from the slope of urinary excretion rate ($\Delta Au/\Delta t$) versus the plasma concentration at the midpoint of the urinary collection period.

Pharmacokinetic parameters from bleomycin courses studied with a cumulative cisplatin dose of < 300 mg/m² (n = 3) were compared to parameters from bleomycin course studied with a cumulative cisplatin dose of > 300 mg/m² (n = 3). A P value of < 0.05 was considered significant.

RESULTS

The plasma concentration-time data were adequately described by a two-compartment open model. The six bleomycin courses were divided into courses administered with a cumulative cisplatin dose of < and > 300 mg/m². This cisplatin dosage was chosen because no differences in pharmacokinetic parameters were noted until > 300 mg/m² of cisplatin was administered. Bleomycin CL_T was significantly reduced from 39 to 18 ml/min/m² when the cumulative cisplatin dose was > 300 mg/m² (P = 0.02). The terminal-phase half-life (β-half-life) also increased from 4.4 to 6.0 hrs, but this difference was not statistically significant (P = 0.52). In Patient 2, CL_R decreased from 30.0 ml/min/m² during the first bleomycin course to 8.2 ml/min/m² during the eighth bleomycin course. No other pharmacokinetic parameter changed significantly (P > 0.1) when bleomycin courses studied at a cumulative cisplatin dose of < 300 mg/m² of cisplatin were compared to those courses when the patient had received > 300 mg/m².

Table 1 shows the cumulative cisplatin dosage, renal function tests, and bleomycin β-half-life, CL_T, and CL_R for each course. Linear regression was performed to assess relationships between estimated creatinine clearance, biologic half-life, CL_T, and cumulative cisplatin dosage. As expected, creatinine clearance decreased as the cumulative cisplatin dosage increased (r = 0.85), although this correlation was not statistically significant (P = 0.07). Cumulative cisplatin dosage also correlated with bleomycin β-half-life (r = 0.83, P = 0.09) and CL_T (r = 0.85, P = 0.07), but these changes in bleomycin elimination were probably caused by cisplatin-induced decreases in creatinine clearance. This is supported by similar correlations between bleomycin elimination (β-half-life, CL_T) and creatinine clearance.

DISCUSSION

Bleomycin is primarily excreted unchanged in the urine, with a CL_T in adults, with normal renal function of 40–50 ml/min/m² (5). Although older children and adolescents eliminate bleomycin similar to adults, young children (< 3 years old) eliminate bleomycin more rapidly than adults (6).

Cisplatin therapy causes dose-limiting nephrotoxicity. Although the incidence and severity of this side effect are reduced by vigorous hydration and mannitol diuresis, pathologic studies show that renal damage persists even though serum creatinine or BUN values return to normal (7). Furthermore, a study in children who received the same dose schedule, length of infusion, and hydration regimen as the two patients presented in this report

shows that serious renal dysfunction can occur in children who receive long-term cisplatin therapy (8). In our study, as the cumulative cisplatin dosage increased, creatinine clearance decreased, and these reductions in creatinine clearance were associated with delayed bleomycin elimination. It is possible, however, that cisplatin reduced bleomycin CL_T by nonrenal mechanisms.

The clinical implications of this drug interaction are unclear. Severe toxicity has been observed in two other patients with renal dysfunction who received standard doses of bleomycin (9,10). We did not observe enhanced bleomycin-related toxicity in our two patients, probably because of differences in dose schedule. Our patients received single bleomycin doses every 3–4 weeks, whereas the two patients who experienced severe toxicity received daily bleomycin (8–10 mg/m^2) for at least 6 days.

Further studies are needed to precisely determine the cumulative cisplatin dose that produces changes in bleomycin elimination. As shown in Patient 1, commonly used tests of renal function such as serum creatinine or BUN are probably insensitive predictors of changes in bleomycin elimination.

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Phase II Trial of 4′-Epi-Doxorubicin in Patients With Non-Small Cell Lung Cancer[1,2]

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4′-Epi-doxorubicin (4′ED) is a doxorubicin derivative that differs from the parent compound by the epimerization of the hydroxyl group at the 4′ position (1). In preclinical testing, it demonstrated a broad spectrum of activity (1,2). A phase I study of 4′ED indicated that at an iv dose of 70–85 mg/m^2 every 21 days, consistent but acceptable myelosuppression was produced, with no major nonhematologic toxic effects (3,4). We have conducted a phase II study of this agent in patients with non-small cell lung cancer (NSCLC).

PATIENTS AND METHODS

Thirty-seven patients (table 1) with stage III M_1 NSCLC were entered in the study. Eligibility criteria included the presence of measurable lesions, PS ≥ 50, normal hepatic, renal, and cardiac functions, wbc count ≥ 4000/mm^3, and platelet count ≥ 100,000/mm^3. 4′ED was administered iv every 21 days (table 2). Standard response criteria were employed.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Age in yrs	
Median: 60	
Range: 33–70	
Performance status (PS)	
80–90	11
50–70	23
Prior treatment	
None	11 (8 with PS ≥ 70)
Chemotherapy	9
Radiotherapy	3
Chemotherapy and radiotherapy	11

RESULTS

Thirty-four of the 37 patients received an adequate trial. The three inadequately treated patients had disease progression without hematologic toxicity after receiving one dose. One patient, previously treated with radiotherapy only, had a partial response lasting 5 months. Three patients had stable disease (11+, 14+, and 17+ months, respectively). All other patients had disease progression.

TOXICITY

Leukopenia was the major toxic effect observed. The median wbc count nadir, generally seen 10–14 days after administration, was 3400/mm^3, with a range of 300–17,000/mm^3. Thrombocytopenia was not a problem. Alopecia occurred in all patients, and there was no muco-

TABLE 2.— Drug regimen

Criteria	4′ED dose
Initial PS > 70	85 mg/m^2
Initial PS 50–70 or prior chemotherapy	65 mg/m^2
If wbc count < 4000/mm^3 or platelet count < 100,000 /mm^3 on Day 21	Hold dose
If wbc count nadir > 4000 /mm^3 or platelet count nadir > 150,000/mm^3	Escalate next dose by 15 mg/m^2
If wbc count nadir < 2500/mm^3 or platelet count nadir < 75,000 /mm^3	Reduce next dose by 15 mg/m^2

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sitis or drug-related hepatic or renal toxicity. No patient developed clinical evidence of congestive heart failure. Four patients receiving total doses > 500 mg/m² (range, 550-1100) underwent serial radionuclide ventriculograms, with no diminution in resting ejection fraction. Patients received a median of two courses of 4′ED.

DISCUSSION

The combined results of several phase II studies of doxorubicin in patients with NSCLC have shown an overall major response rate of 9% (range, 0%-24%) (5). In this trial of 4′ED, with only one of 34 adequately treated patients achieving a major response, the predicted true response rate is ⩽ 15% ($P = 0.05$). We conclude that 4′ED, in the present dose and schedule, has only marginal activity in patients with NSCLC.

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Phase II Study of Hycanthone in Patients With Advanced Colorectal Carcinoma[1,2]

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Hycanthone, an effective antischistosomal agent, is a thioxanthone derivative and a hydroxymethyl analog urinary excretion product of lucanthone, which can also be obtained by hydroxylation of lucanthone in the presence of Aspergillus sclerotiorum (1). The planar aromatic nucleus of hycanthone, containing a keto group and a diethylamino moiety in the side chain, are structural features common to known antileukemic agents. Hycanthone has demonstrated activity against murine leukemias L1210 and P388 (2) related to its property as a DNA intercalator (nearly as strong as daunorubicin) (3). Hycanthone is mutagenic in mammalian cells and teratogenic in mice. Primary toxic effects in monkeys and dogs included CNS neurotoxicity, persistent anorexia, and hepatotoxicity. No significant myelotoxicity was seen (4). Side effects of hycanthone administered to humans for antischistosomal therapy have been primarily nausea and vomiting and occasional toxic hepatitis. Phase I studies of hycanthone reported dose-limiting toxic hepatitis at dose levels $\geq$ 80 mg/m^2 iv daily $\times$ 5 (5,6). No definite tumor regressions were seen. This phase II study was carried out to determine whether hycanthone has antineoplastic activity in human colorectal carcinoma and to obtain more data regarding the toxicity of hycanthone.

MATERIALS AND METHODS

Eligibility criteria for this study included histologic confirmation of unresectable or metastatic colorectal adenocarcinoma, measurable tumor, Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–3, adequate nutrition, and informed consent. Treatment was delayed at least 4 weeks after prior radiation therapy, chemotherapy, or major surgery. Patients were excluded from this study if they had leukocyte or platelet counts less than normal, any elevation of direct bilirubin, serum creatinine > 1.9 mg/100 ml, or SGOT or alkaline phosphatase twice the normal rate. Based on phase I observations, hycanthone was diluted in 5% dextrose in water to 1 mg/ml, administered at a dose of 60 mg/m^2 infused iv for 20 minutes daily on each of 5 consecutive days, and was repeated every 4 weeks in patients with objectively stable disease. Hycanthone treatment was discontinued in patients with disease progression. After treatment, SGOT levels were determined once weekly, and, at 4 and 8 weeks, indicator lesions were remeasured, and all pretreatment laboratory studies were repeated.

RESULTS

Thirty patients were entered in this study, including 11 males and 19 females with a median age of 60 years (range, 21–77). Thirteen patients had received prior single-agent or combination chemotherapy (5-FU, tegafur, and semustine, six each; Baker's antifol, vincristine, and maytansine, one each). Each patient was ambulatory, 20 had a good ECOG PS of 0–1, while only two had an ECOG PS of 3. The major toxicity associated with hycanthone was hepatic. Sixteen of 29 patients had asymptomatic increases in SGOT, ranging from one to five times pretreatment values. Three patients had SGOT increases of greater than five times pretreatment values, including two who had therapy interrupted on Day 4 after SGOT values elevated to 418 and 640 mg/100 ml, respectively, although neither patient developed jaundice. There was no evidence of cumulative hepatotoxic reactions in the 11 patients who received multiple (two to five) courses of hycanthone. Nonhepatic toxic reactions to hycanthone treatment in 29 patients included nausea (72%) and vomiting (52%) which were usually mild and limited to the 5-day course of treatment. Two patients each reported anorexia or diarrhea, and one reported headache. None of the

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30 patients treated with hycanthone showed evidence of objective regression of metastatic disease. Eighteen patients showed objective progression at 4 weeks, six at 8 weeks, four at 12 weeks, one at 16 weeks, and another at 20 weeks. Results are shown in table 1.

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Total eligible	30
Sex	
Male	11
Female	19
Median age in yrs (range): 60 (21-77)	
ECOG PS	
0-1	20
2	8
3	2
Response (at 8 wks)	
Complete/partial	0 (0)
Stable disease	6 (20)
Progressive disease	24 (80)
Toxicity (total, 29 patients)	
Nonhematologic	
None	6 (21)
Nausea	21 (72)
Vomiting	15 (52)
Anorexia	2 (7)
Diarrhea	2 (7)
Headache	1 (3)
Hepatotoxicity	
None	10 (34)
SGOT elevated 1-5 times pretreatment	16 (55)
SGOT elevated > 5 times pretreatment	3 (10)

DISCUSSION

In our phase II study, hycanthone produced mild, clinically tolerable hepatic and gastrointestinal toxicity, but appeared devoid of clinical activity against metastatic large bowel carcinoma. It is of interest that other DNA intercalators (ie, doxorubicin, daunorubicin) have also lacked significant therapeutic activity against colorectal carcinoma.

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High-Dose Intermittent Iv 5-FU and Melphalan in Advanced Colorectal Carcinoma[1]

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A previous study of iv 5-FU and oral melphalan in 25 patients with advanced colorectal carcinoma produced no serious toxicity and a 24% objective response rate (1). It is now known that iv administration of melphalan is more reliable than oral administration, and clinical studies using high-dose intermittent scheduling of chemotherapy have indicated the potential benefit of less clinical and marrow toxicity without compromise of the antitumor effect (2). A further study has been conducted using a 12-hour iv infusion of 5-FU followed in 8 hours by an iv bolus of melphalan.

Patients entered into the study had measurable metastases of histologically proven colorectal carcinoma; Eastern Cooperative Oncology Group performance status of 0, 1, or 2; and adequate bone marrow and renal functions. All patients gave informed consent. None had received prior chemotherapy. Tumor response was assessed using standard Eastern Cooperative Oncology Group criteria (3).

Fourteen patients received one to seven courses (median, three) of treatment. Nausea and vomiting, which rarely occurred during 5-FU infusion, was common about 4 hours after the iv bolus of melphalan. Patients generally remained free of clinical toxicity between courses of chemotherapy. Patient characteristics and response to therapy are shown in table 1.

This drug regimen, although designed according to optimum principles of dose, route, and scheduling, proved ineffective and unacceptably toxic. According to Gehan (4), a study of 14 patients with no objective responses represents a 95% probability that the true response rate is not > 20%. Combining the results of this study with our previous results and those of others (1) indicates an overall objective response rate of 12.8% for nine of 70 patients; this is inferior to the generally accepted response rate of 21% achieved with 5-FU alone (5). The significantly greater toxicity of 5-FU plus melphalan makes this combination of drugs unsuitable for palliative therapy of advanced colorectal carcinoma.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Mean age in yrs (range): 56 (28–77)	
Males/females	8/6
Primary site	
Colon	11
Rectum	3
Metastatic sites	
Liver	11
Local and peritoneal	6
Other visceral	2
Multiple sites	6
Drug schedule	
5-FU, 50 mg/kg/4 wks	
Melphalan, 0.3 mg/kg/4 wks	
Dose modification	
2/3 if marrow recovery > 4 wks	
Toxicity	
Nausea and vomiting	10
Thrombophlebitis	6
Alopecia	2
Stomatitis	1
Diarrhea	1
No clinical toxicity	3
Leukopenia < 2000 cells/mm^3	2
Leukopenia, 2000–4000 cells/mm^3	5
Thrombocytopenia < 25,000 cells/mm^3	2
Thrombocytopenia, 50,000–100,000 cells/mm^3	1
No marrow suppression	6
Chemotherapy-related deaths	0
Response	
Complete and partial	0
Stable	3
Disease progression	11
Median survival in mos (range): 7 (1–18)	

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Treatment of Advanced Non-Small Cell Lung Cancer With Bisantrene[1,2]

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Bisantrene, a new synthesized anthracenedicarboxaldehyde hydrazone, was found to have significant antitumor activity in experimental animal systems (1). In L5178Y mouse lymphoma tissue culture cells, bisantrene was a potent inhibitor of both RNA and DNA synthesis (1-3). When compared on an equimolar basis, bisantrene was approximately three times more potent than doxorubicin in inhibiting the incorporation of ^{3}H-uridine into RNA and four times more potent in inhibiting the incorporation of ^{3}H-thymidine into DNA.

Twenty-one patients with measurable histologically confirmed advanced non-small cell lung cancer were entered into this study. Patient characteristics are outlined in table 1. Eleven patients had received previous chemotherapeutic regimens (range, two to seven drugs). Four of these patients had received doxorubicin in combination with other cytotoxic drugs. Eighteen patients had poor performance status. Bisantrene was administered at an initial dose of 260 mg/m^2 in 500 ml of 5% dextrose in water and was given iv over 60 minutes. Subsequent therapy was repeated every 3 weeks with dosage modification based on toxicity.

Twenty treated patients were evaluable for toxicity and response. One patient was not evaluable due to early, unexplained death on Day 7 of the first bisantrene treatment. Forty-five courses of bisantrene were given to the 20 evaluable patients, with a median of two courses (range, one to five) per patient. There were no objective responses. Calculated from the first day of treatment, eight patients demonstrated progression of disease within 6 weeks; the remaining 12 patients had initial disease stabilization for 9-15 weeks followed by disease progression.

Leukopenia, the only hematologic toxic effect in this study, occurred in 11 of the 20 evaluable patients. Among these 11 patients, four had had no prior cytotoxic therapy. During the study, seven patients experienced a wbc count nadir between 2100 and 3000/μl, and the additional four patients had a wbc count nadir between 1200 and 2000/μl. In all of the courses that induced leukopenia, the median number of days to recovery (wbc count > 3000/μl) was 7 (range, 3-12), calculated from the nadir day. For all 45 evaluable courses, the median wbc count nadir was 3700/μl (range, 1200-6400). Nonhematologic toxic effects were

TABLE 1.—Characteristics of treated patients*

Characteristics	Prior chemotherapy	No prior chemotherapy
Entered patients	11	10
Evaluable patients	10	10
Age in years		
Median	54	64.5
Range	32-72	36-78
Male:female	9:2	10:0
Performance status†		
0/1	2	1
2/3	9	9
Prior radiotherapy	2	4
Histology		
Adenocarcinoma	7	3
Squamous cell carcinoma	4	5
Large cell anaplastic carcinoma	0	2
Extent of disease‡		
Limited:extensive	3:8	5:5
No. of bisantrene courses		
Median	2	1
Range	1-5	1-3

* Unless otherwise indicated, values = No. of patients.

†Cancer and Leukemia Group B criteria.

‡Disease confined to 1 lung, including mediastinal and ipsilateral supraclavicular nodes, was classified as limited. Disease beyond these confines was classified as extensive.

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mild and consisted of nausea and vomiting (three patients) and hypotension (two) which could be eliminated by lengthening the infusion time from 1 to 2 hours. There was no evidence of drug-induced phlebitis or anaphylactoid, cardiac, renal, or hepatic toxicity. While bisantrene at this dose and schedule is well-tolerated, it shows < 20% activity with 95% confidence limits against advanced non-small cell lung cancer in patients with poor performance status.

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Phase II Study of High-Dose Amsacrine (AMSA) and Autologous Bone Marrow Transplantation in Patients With Refractory Metastatic Breast Cancer[1,2]

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Amsacrine (AMSA) is a recently developed antitumor agent that has shown modest activity against breast cancer (1-3), with myelosuppression being the major limitation to dose escalation. We have previously reported the feasibility of high-dose AMSA followed by autologous bone marrow transplantation (ABMT) in a few patients with solid malignancies and have defined the dose-limiting toxicity (4). Encouraged by the dose-responsiveness of adult acute leukemia to AMSA (5), we began a phase II study of high-dose AMSA followed by ABMT in previously treated patients with metastatic breast cancer.

PATIENTS AND METHODS

To be eligible for the study, patients were required to have pathologically defined breast cancer with evaluable metastatic lesions, normal peripheral cell counts (wbc count $\geq 4000/\mu l$ and platelet count $\geq 100 \times 10^3/\mu l$), normal hepatic and renal functions (serum bilirubin < 2 mg/dl, SGOT < 40 IU/ml, and serum creatinine < 1.5 mg/dl), and, at the time of bone marrow collection, no bone or bone marrow disease. The patient characteristics are summarized in table 1. Unless they developed progressive disease after the first course of AMSA, all patients received two courses of high-dose AMSA. In the first 11 patients treated, AMSA was administered at a dose of 200 mg/m^2/day × 3 and repeated at 3-week intervals, with the second course given at the same dose level. Because no significant nonhematologic toxicity was observed at this initial dose of AMSA, the next five patients received 250 mg/m^2/day × 3/course. ABMT was carried 48-72 hours after completion of chemotherapy. Patients who responded after two courses were given lower doses of AMSA as maintenance therapy, after full hematologic recovery. The starting maintenance dose was 30 mg/m^2/day × 3. Subsequent doses of maintenance AMSA were adjusted in accordance with the degree of previous

TABLE 1.—Patient characteristics and treatment results

Characteristic	No. of patients
Total evaluable	16
Age (yrs)	
Median: 48	
Range: 32-69	
Performance status	
0-1	10
2-3	6
Dominant site of disease	
Soft tissue	5
Visceral	8
Bone	3
Prior therapy	
Chemotherapy	16
Median No. of regimens: 2.5 (range, 1-6)	
Radiation therapy	8
Endocrine therapy	11
Response	
Complete	0
Partial	2 (12.5%)
Hematologic toxicity	
Severe thrombocytopenia ($< 20 \times 10^3$ cells/μl)	16
Median No. of days: 4	
Severe leukopenia (< 500 cells/μl)	16
Median No. of days: 12	
Hematologic recovery	
Neutrophil count ($> 1000/\mu l$): median, 24 days (range, 15-31) after ABMT	16
Platelet count ($> 100 \times 10^3/\mu l$): median, 17 days (range, 11-27) after ABMT	16

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myelosuppression, and were given at 3-week intervals. Standard criteria for response assessment were utilized.

RESULTS

Two patients (one with lung and one with bone involvement) achieved partial remissions for 11 and 7 months, respectively. Two patients had stable disease for 7 and 4 months, respectively, and 12 had progressive disease.

Both high-dose AMSA schedules produced profound myelosuppression (table 1). There were four episodes of septicemia (bacterial, three; fungal, one) and ten episodes of fever of unknown origin associated with neutropenia during 25 courses of high-dose AMSA. All patients had prompt hematopoietic recovery after ABMT (table 1). The nonhematologic toxicity mainly involved the gastrointestinal tract. Nausea and vomiting were universal. Eight patients developed a mild nonbloody diarrhea. However, mucositis was the dose-limiting toxic effect, occurring in ten of 25 courses. Four episodes of severe mucositis occurred in five patients treated at the higher dose, while only one of six episodes was severe at the lower dose. No cardiac or neurologic abnormalities were noted.

DISCUSSION

The data in this study suggest that high-dose AMSA may not be more effective than conventional doses in the treatment of refractory metastatic breast cancer. Mucositis is the limiting toxicity at the doses used in this study. Further clinical trials are still needed to define the role of high-dose chemotherapy and ABMT in solid malignancies refractory to more conventional doses.

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Phase II Evaluation of Phenesterin in Patients With Advanced Ovarian Cancer[1,2]

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Phenesterin was synthesized by Larionov et al (1) as a mustard ester of cholesterol with chlorophenacyl. Activity was demonstrated against rat and mouse mammary carcinomas and brain tumors, including Sarcoma 45, Walker 256 carcinosarcoma, and the RS-1 alveolar liver carcinoma (1,2). In a phase I study, Ansfield et al (3) treated 30 patients with metastatic or primary inoperable breast cancer who had previously been treated with hormone or cytotoxic compounds. Only one patient showed evidence of significant regression. An Eastern Cooperative Oncology Group (ECOG) phase II study compared two doses of phenesterin in 222 evaluable patients with measurable progressive metastatic cancer (4). The greatest activity was seen in ovarian cancer; there were five objective responses, including two complete remissions (CR) among 14 patients, with one CR lasting > 2 years. Because of these preliminary findings, a phase II study to further define the effect of phenesterin against ovarian carcinoma was performed by member institutions of the ECOG.

Seventy-nine patients with advanced ovarian cancer were randomized to receive either phenesterin or mitoguazone. The patients who received mitoguazone will be reported separately. Phenesterin was administered to 38 patients with advanced ovarian cancer, of whom 37 were evaluable. Patient characteristics are shown in table 1. All patients had failed prior chemotherapy, and four had received prior hormonal therapy. Each patient received 80 mg/m^2 orally twice/week. Standard ECOG criteria for dose reduction and response were employed. An adequate trial was considered to be 8 weeks of therapy or evidence of disease progression. Total dose ranged from 500 to 2600 mg (mean, 1200).

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Performance status	
Ambulatory	20 (54)
Nonambulatory	17 (46)
Age in yrs	
< 60	20 (54)
⩾ 60	17 (46)
Stage	
III	21 (57)
IV	16 (43)
Grade	
Moderately well-differentiated	18 (48)
Poorly differentiated	14 (38)
Unknown	5 (14)
Weight loss	
No	19 (51)
Yes	17 (46)
Not reported	1 (3)
Prior radiotherapy	
No	27 (73)
Yes	10 (27)
Prior chemotherapy (> 2 agents)	37 (100)
Time from diagnosis to on-study	
⩽ 12 mos	17 (46)
> 12 mos	20 (54)

RESULTS

Phenesterin was well-tolerated. Moderate gastrointestinal symptoms occurred in 20% of the patients, and mild hematologic toxic effects occurred in 41%. One patient

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had severe thrombocytopenia. No other clearly drug-related toxic effects were observed. There were no CRs. One patient (2.7%) had a partial response with a > 50% reduction of pelvic mass, which lasted for 4 weeks. Of the remaining 36 patients, only seven did not have evidence of disease progression by the time they were removed from the study. The median survival time was only 8.7 weeks for this heavily pretreated group of patients.

CONCLUSION

Our results do not confirm the findings of our prior phase II investigation of phenesterin, in which some activity against ovarian cancer was found in a small patient sample. We conclude that phenesterin given in this dose schedule had minimal to no activity in heavily pretreated patients with advanced ovarian carcinoma.

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Acute Promyelocytic Leukemia Following ABVD (Doxorubicin, Bleomycin, Vinblastine, and Dacarbazine) and Radiotherapy for Hodgkin's Disease[1]

Second malignancies, especially acute nonlymphocytic leukemia (ANLL), are being reported with increasing frequency following successful treatment of Hodgkin's disease (HD) (1). In particular, patients treated with both extensive radiotherapy and MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) appear to be at highest risk for developing ANLL (1,2). Potentiation of the carcinogenic effect of radiation by alkylating agents and/or procarbazine has been postulated as one possible causative mechanism of leukemogenesis (1,2). Recently, Valagussa et al (2) reported on the lack of carcinogenic potential of ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) as compared to MOPP when employed either alone or in combination with radiotherapy in the treatment of HD. We report here a patient who developed acute promyelocytic leukemia (APL) following treatment with ABVD and radiotherapy for HD.

CASE REPORT

A 45-year-old white female was diagnosed with stage IIIA mixed cellularity HD in December 1979. She was treated with 6 months of ABVD at the dose schedule proposed by the group in Milan (2), and a complete remission was achieved in July 1980. In December 1980, recurrent disease was suspected because of the appearance of enlarged left cervical nodes and the positivity of an abdominal computerized tomographic scan demonstrating the presence of para-aortic lymphadenopathy. She was treated with total nodal radiotherapy with disappearance of measurable disease and remained well until December 1981 (24 months after initial therapy) when she noticed increasing pallor, fatigue, and easy bruising. Physical examination was unremarkable except for the presence of scattered purpura over the lower extremities. The hematocrit was 25%; the hemoglobin was 7.9 g/dl; the wbc count was 1000/mm^3, with 92% lymphocytes and 8% leukemic promyelocytes; and the platelet count was 48,000/mm^3. The bone marrow was hypercellular and showed an excess (> 80%) of hypergranular blast cells, most of which contained characteristic bundles of large Auer rods. By morphologic and cytochemical criteria, the picture was that of APL (M-3 variant according to the FAB classification). The coagulation profile was normal except for a low fibrinogen (80 mg/100 ml) and strong positivity of the ethanol-gelatin test. The patient denied permission for further clinical and laboratory investigations, refused chemotherapy, and died at home 2 weeks later of intracranial hemorrhage.

DISCUSSION

ANLL is a well-recognized late complication of curative treatment of HD. Although ANLL has been reported following different modalities of therapy, the highest incidence has occurred after extensive radiotherapy combined with MOPP, particularly in patients who began treatment of their disease at ≥ 40 years of age (3). In a recent retrospective analysis of 1032 cases of HD in Milan, Valagussa et al (2) found that 3% and 7.9% had developed ANLL and other malignancies, respectively, within 10 years after initial therapy. Interestingly, ANLL was observed only in patients whose treatment included chemotherapy programs containing alkylating agents and/or procarbazine (MOPP or MOPP-like regimens). In contrast, no cases of ANLL or other secondary neoplasms were observed in those patients who received ABVD, a regimen devoid of the above-mentioned drugs, with or without radiotherapy. This observation further supports the hypothesis that mechlorethamine and procarbazine are primarily involved in the promotion of the carcinogenic event (1,2). Although hardly surprising because the drugs in ABVD have also been proved to be carcinogenic and immunosuppressive in animals (4), this case of ANLL developing after combined modality therapy for HD presents two major points of interest. First, this is the first case of a secondary malignancy occurring in HD after treatment with ABVD reported in the literature so far. Radiation-induced leukemias have a rather long latency interval (> 5 years); thus, it seems unlikely that radiotherapy was responsible for the occurrence of APL in this patient since the interval from radiation to APL was only 1 year. And second, APL has rarely been associated with the course of HD or any other hematologic and nonhematologic neoplasm treated with radiotherapy and/or chemotherapy (5). Most patients with secondary ANLL have had acute myelocytic or myelomonocytic leukemia, although erythroleukemia and acute monocytic leukemia have also been described (1). In view of the supposed low

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carcinogenic potential of ABVD and considering the peculiar morphology of the leukemic blasts, the possibility of a chance association between HD and APL cannot be ruled out in this patient. More observations and longer follow-up of patients with HD treated with a therapeutic program, including ABVD, will permit a better assessment of the carcinogenic effects of this regimen as compared to MOPP or MOPP-like combinations.

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Vincristine Neuropathy With Bowel and Bladder Atony, Mimicking Spinal Cord Compression

Vincristine is a vinca alkaloid that is effective in treating hematologic malignancies and some solid tumors. A vincristine-treated patient developed severe peripheral neuropathy and incontinence of bowel and bladder, with electromyogram (EMG) evidence of denervation of the anal sphincter. Chemotoxicity may lead to incontinence, as may nerve root or epidural spinal tumor.

CASE REPORT

A 60-year-old man with asymptomatic chronic myelogenous leukemia diagnosed in 1977 was treated with hydroxyurea, supplemented in 1978 by two courses of cytarabine plus 6-thioguanine. Blast crisis in 1981 was treated with vincristine (2 mg iv every 7-10 days for eight doses) plus prednisone (100 mg daily orally, later tapered to 5 mg daily). By December 1981, he experienced paresthesias in his feet and hands which later improved somewhat. When he relapsed 1 month later, he had symmetrical mild weakness and hypoflexia. He was treated with prednisone (60 mg daily orally) plus a weekly dose of vincristine (2 mg iv) for 2 successive weeks. Distal weakness and stocking-glove paresthesias progressed rapidly after the second dose of vincristine. One week later, rectal soiling and frequent bowel movements occurred, with urinary incontinence 1 day later. He had no back or abdominal pain, bloating, or orthostatic symptoms. Muscle stretch and bulbocavernous reflexes and Babinski responses were absent; anal wink was present but diminished. Sensation to pinprick was diminished in a stocking-glove distribution to the midthighs and mid-forearms; saddle sensation was normal. Rectal tone was absent. Strength was symmetrically reduced and was more pronounced distally.

Abdominal and lumbosacral spine roentgenograms were unremarkable. Cerebrospinal fluid, complete myelogram, and cranial computerized tomography were normal. Postvoid catheterized residual urine volume was 900 ml; urinary incontinence, therefore, was probably due to overflow. EMG in the extremities showed widespread polyphasic potentials and diminished interference pattern that were more pronounced distally. The anal sphincter EMG also showed polyphasic potentials. Nerve conduction velocities were slowed: tibial nerve, 28 m/sec; median and ulnar nerves, 45-48 m/sec.

Further chemotherapy was withheld. One week later, the patient was partially continent of stool. Weakness and hypesthesia were stable. Two weeks later, the patient required only intermittent catheterization. On voiding cystourethrogram, he had bladder sensation at 700 ml and a postvoid residual of 150 ml. Four weeks after the onset of severe neurologic symptoms he was fully continent. He ambulated with a walker; strength was 4-/5 in the legs. Sensation to pinprick was diminished only in the feet; his reflexes remained absent.

DISCUSSION

Neuropathy develops during vincristine therapy in most patients, usually limited to early loss of muscle stretch reflexes and distal paresthesias. Weakness, when it occurs, is more marked distally than proximally and is more evident in the legs than in the arms. Toe and foot extensors are typically most affected, producing a slap-

ping gait (1,2). Cranial nerve signs may occur in 10% of the treated patients (1). Autonomic signs (1-3) include constipation in most patients and ileus in many; orthostatic hypotension and urinary retention have also been reported. Myenteric plexus involvement has been documented in experimental vinblastine neuropathy (4). The peripheral neuropathy thus may affect cranial, autonomic, motor, and sensory nerves. The EMG typically shows denervation consistent with the weakness; early changes include diminution of motor nerve action potential amplitude (2). Mildly slow nerve conduction velocities suggest a primarily axonal neuropathy, which pathologic studies confirm. Lumbar punctures were normal in one report (1). Although patients generally improve when vincristine is stopped, more severely affected patients may not; improvement has also been noted during ongoing treatment (2). Seizures and inappropriate secretion of antidiuretic hormone are other neurologic toxic effects that are rarely related to vincristine therapy.

This patient had the rapid onset of bowel and bladder incontinence coincident with worsening of a severe but characteristic vincristine neuropathy. Anal sphincter denervation, documented by EMG, has not been reported previously with vincristine neuropathy. Normal myelography excluded spinal cord compression. Disseminated meningeal leukemia was unlikely with normal cerebrospinal fluid cytology, absence of cancer peripherally, and neurologic improvement while off antitumor therapy. Rarely, if ever, is such rapid clinical improvement seen in patients with neuropathy as a remote effect of cancer (5). Although incontinence sparks concern over possible spinal cord compression, anal sphincter denervation with incontinence, rather than constipation, may occur with overflow urinary incontinence in vincristine toxicity without other signs of autonomic neuropathy.

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April 13, 1983

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High-Dose Medroxyprogesteroneacetate (MPA) in Advanced Breast Cancer

Vienna, Austria, August 28–September 2, 1983

Sponsored by the Association for Interdisciplinary Oncology of the German Cancer Society, the goal of this symposium is to extensively evaluate and assess the current experience with high-dose MPA in metastatic breast cancer. The experimental and clinical background of high-dose MPA will be presented in two main lectures. A general and round table discussion will concentrate on the following topics: (*a*) proposed mechanism of action of high-dose MPA; MPA blood levels: determination and correlation to tumor response; (*b*) monitoring of effective MPA dosage by cortisone and prolactin measurement; (*c*) MPA resistance; (*d*) MPA in drug combinations; (*e*) bone marrow protection by MPA; and (*f*) prevention of MPA-induced side effects, especially Cushing, phlebitis, and water retention.

The consensus discussion is to answer the following questions: what is high-dose MPA?; Indications for and positioning of high-dose MPA in breast cancer; How to monitor safe and effective dosage; Role of MPA in combination chemotherapy regimens; and, Role of MPA in adjuvant therapy.

Short free communications, maximum 5 minutes, relevant to the main topics may be included in the final program and publication. The communications must be announced before *July 31, 1983*, indicating author and title of the paper.

The symposium will be published shortly after the congress, including mostly the main lectures, invited communications, and selected free communications, as well as summaries of the discussion and the consensus meeting. Participants who wish to have their short communication published are requested to submit their manuscript of, at most, four typewritten pages including tables, figures, and literature references to the symposium chairmen at the meeting. The final program including announced short communications will be distributed at the beginning of the congress.

For more information, contact: G. A. Nagel, MD, Medical University Clinics, Robert-Koch-Str. 40, D-3400 Goettingen, W Germany.

Aminogluthethimide as an Aromatase Inhibitor in the Treatment of Cancer

Vienna, Austria, August 28–September 2, 1983

Sponsored by the Association for Interdisciplinary Oncology of the German Cancer Society, the goal of this symposium is to review recent developments in this field and to introduce aromatase inhibition as a treatment modality for malignant tumors, especially breast cancer. Experimental and clinical data will be reviewed by invited speakers.

Short free communications, maximum 5 minutes, relevant to the main topics may be included in the final program and publication. The communications must be announced before *July 31, 1983*, indicating author and title of the paper.

The symposium will be published shortly after the congress, including mostly the main lectures, invited communications, and selected free communications, as well as summaries of the discussion and the consensus meeting. Participants who wish to have their short communication published are requested to submit their manuscript of, at most, four typewritten pages including tables, figures, and literature references to the symposium chairmen at the meeting. The final program including announced short communications will be distributed at the beginning of the congress.

For more information, contact: G. A. Nagel, MD, Medical University Clinics, Robert-Koch-Str. 40, D-3400 Goettingen, W Germany.

Twentieth National Reticuloendothelial Society Meeting

Portland, Oregon, October 9–12, 1983

The Scientific Program Chair for this meeting, to be held at the Portland Hilton Hotel, is Dr. Robert I. Mishell, University of California, Berkely, and the Local Host Chair is Dr. Jane M. Siegel, University of Oregon Health Science Center, Portland. The scientific sessions will consist of a workshop, three

symposia, submitted papers, poster presentations, and a keynote address.

There will be two special award competitions held this year for submitted abstracts that will be judged on the basis of their scientific merit and their presentation at the national meeting. The Presidential Awards will be for students, and the Young Investigator Award will be for investigators under age 36. First prize for both competitions is a travel stipend to the International Congress of the Reticuloendothelial Society to be held at Mount Fuji, Japan, in 1984.

For more information, contact; Reticuloendothelial Society, c/o Dr. Sherwood M. Reichard, Medical College of Georgia, Augusta, GA 30912.

Current Concepts in Cancer Therapy, II

St. Louis, Missouri, November 3–5, 1983

This meeting will be presented by the Department of Surgey and the Office of Continuing Medical Education, Washington University School of Medicine in St. Louis. The program chairman is Marc K. Wallack, MD. The meeting will be accredited for 17 hours AMA Category 1, AAFP Prescribed, and AOA.

For further information, contact: Loretta Giacoletto, Office of CME, Washington University School of Medicine, Box 8063, 600 S Euclid, St. Louis, MO 63110 (telephone 314-454-3873).

Cincinnati Cancer Conference: Gastro Intestinal Malignancies

Cincinnati, Ohio, November 4–5, 1983

This conference is sponsored by the Bethesda Hospital and Deaconess Association, and the University of Cincinnati.

For further information, contact: Thomas O'Connor, Medical Staff Education, Bethesda Hospital, 619 Oak St, Cincinnati, OH 45206.

Third Congress of the European Society of Urological Oncology and Endocrinology

Rome, Italy, November 24–26, 1983

The main topics of discussion at this meeting, given as lectures by experts in the field, include: immunofluorescence studies of steroid receptors, etiology and epidemiology of prostatic tumors, biochemical aspects of etiology and epidemiology in prostatic tumors, genetic control and cell kinetics of cancer cell, immunotherapy of kidney tumors, experimental basis and clinical results, and monoclonal antibodies in urology.

Registration fees, including membership to Scientific Session, Congress documents, abstracts, simultaneous translation equipment, and social events is $50 and $25 for members.

For further information, contact: Franco Di Silverio, MD, President, Third Congress European Society of Urological Oncology and Endocrinology, c/o ACTA MEDICA s.r.l., Viale Parioli, 3-Rome, Italy.

International Symposium of the Ludwig Boltzmann Research-Institute for Infections and Tumors of the Urinary Tract

Vienna, Austria, November 25–26, 1983

Symposia topics include: predictability of cancer chemotherapy with special respect to renal, bladder, prostatic, and testicular tumors; persistent asymptomatic microhematuria; and percutaneous surgery of the kidney—diagnostic and therapeutic approach—indication, technique, risks, and results.

For further information, contact: Dr. P. Porpaczy, c/o Wiener Medizinische Akademie, Alser Straße 4, A-1090, Vienna, Austria. Telephone: (0222) 42 71 65, Cables: Medacad Wien.

FOCA Oncology Fellowships

Brussels, Belgium

Applications are now being accepted for 1983–1984 Fondation pour la Chimiothérapie Anticancéreuse (FOCA) fellowships with the Cancer Center of the University of Brussels. The fellowships are geared toward medical oncologists from the United States who are board-certified in oncology or have equivalent training and want to work one year or longer as a staff member in the Department of Medicine and Clinical Investigation of the Institut Jules Bordet, The Cancer Center of the University of Brussels. The chief of the department is Dr. Jean Klastersky.

Stipend is approximately $35,000–$45,000 (2 million Belgian francs) depending on the exchange rate.

For further information, contact: Professor Henri Tagnon, President of FOCA, Institut Jules Bordet, 1, Rue Héger-Bordet, B–1000 Bruxelles, Belgium (telephone: 02/538.27.66, Telex: 22 773).

Fourth Conference on Human Tumor Cloning

Tucson, Arizona, January 8–10, 1984

This conference will be held at the University of Arizona Cancer Center, and a combination of invited and competitively selected papers, demonstrations, and posters will be presented. Deadline for submission of abstracts is *October 1, 1983*.

Dr. Sydney E. Salmon, and Dr. Jeffrey Trent are the co-chairman for the event.

For more information, contact: Mary Humphrey, Conference Coordinator, University of Arizona Cancer Center, Tucson, AZ 85724 (telephone: 602-626-6044).

Tenth International Cell Cycle Conference

Banff, Alberta, Canada, April 24–28, 1984

The International Cell Cycle Society is holding its tenth conference at the Banff Center. Symposium topics will include the biochemistry and physiology of the cell cycle, chromatin and nuclear structure, genetics of the cell cycle, cell differentiation, fine structure of the cell cycle, and proliferative dormancy and activation.

For more information, contact: Dr. Susan Friedman, The Oncology Research Group, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

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